Preparation of emulsifiers from pea and corn starches using octenyl succinic anhydride modification

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ABSTRACT

The objective of this thesis research was to modify pea starch – a representative pulse starch – using octenyl succinic anhydride (OSA) to prepare emulsifiers for enhanced industrial utilization. Pea starch (PS), normal corn starch (NCS), and waxy corn starch (WCS) were modified with 1%, 3% and 5% OSA (w/w, on a dry basis of starch, db). Native PS consisted of more amylose (41.5%) than NCS (32.9%) and WCS (1.7%). The degrees of substitution (DS), thermal properties, and pasting properties of the derived OS starches were determined. Incorporation of OS groups significantly alter the thermal and pasting properties of the three starches. Gelatinization temperatures and enthalpy changes of the starches were gradually decreased with an increasing level of added OSA. OS starches showed less tendency to retrograde compared to the control starches. OSA modification progressively reduced the pasting temperatures and increased the peak viscosities of the three starches as the DS increased.

The OS starches displayed good capability of lowering canola oil-water interfacial tension (IFT). Canola oil-in-water (O/W) emulsions were prepared with the OS starches to evaluate their emulsifying properties, with gum Arabic (GA) being included as the industry standard for comparison. Droplet-size distributions, zeta potentials, accelerated stability, and storage stability of the resultant emulsions were determined and compared. Fresh emulsions produced with the OS starches showed droplet sizes considerably smaller than that of GA emulsion. NCS and WCS modified with 3% and 5% of OSA and GA exhibited good ability to stabilize O/W emulsions during 28-day storage at 4°C due to steric hindrance and electrostatic repulsion provided by the emulsifier layer on the droplets. However, the droplet sizes of all the OS-PS emulsions increased to some extents during the storage, suggesting the least stability of these emulsions.

Emulsion stability under various environmental conditions was also investigated, including pH 2-7, 0-1.0 M sodium chloride in aqueous medium, and incubation at 90°C for 30 min. The droplet-size distributions and the stability of the emulsions were confirmed by confocal laser scanning microscopy observation. Under the same test condition, the droplet sizes of the generated emulsions were largely in a descending order of OS PS > GA ≥ OS NCS > OS WCS. The results obtained from the thesis suggested that steric repulsion between emulsion droplets provided by the emulsifier layer was the main factor to prevent droplet aggregation and coalescence in OS-starch and GA emulsions and that electrostatic repulsion only partially contributed to stabilize the emulsion systems.

Overall, the ability of the emulsifiers in stabilizing O/W emulsions followed an ascending order of OS PS < GA ≤ OS NCS < OS WCS. The poorer stability of OS-PS emulsions was partly attributed to the higher amylose content and longer amylopectin branch chains of the starch, which could cause stronger re-association and a higher rate of retrogradation between starch molecules to weaken the steric hindrance in the resultant emulsions. Emulsifying properties of the OS starches and stability of the resultant emulsions were generally enhanced with a higher DS, with the most noticeable improvement being observed in OS-PS emulsions.

This study demonstrated the effects of OSA modification on the thermal, pasting and emulsifying properties of different starches. The interrelationships between the molecular structures (*e.g.*, amylose content and degree of substitution), functional properties (*e.g.*, retrogradation rate), and emulsifying capability of the OS starches were also clearly elucidated. The new findings from the thesis can be used to further tailor the structures of OS PS to improve the emulsifying properties for potential industrial uses.

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LIST OF SIMBOLS AND ABBREVIATION

ºC	Degree Celsius
<	Less
>	More
≈	Approximation
≤	Less than or equal to
≥	More than or equal to
ANOVA	Analysis of variance
$AgNO_3$	Silver nitrate
CLSM	Confocal laser scanning microscopy
d	Day
$d_{4,3}$	Volume weighted mean droplet diameter
db	Dry basis of starch
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
DS	Degree of substitution
DSC	Differential scanning calorimetry
DSD	Droplet size distribution
FDA	Food and Drug Administration
g	Gravitational force (9.81 m/s^2)
G’	Storage modulus
G”	Loss modulus
GA	Gum Arabic
h	Hour
ΔH	Enthalpy change
HCl	Hydrochloric acid
I_2	Iodine
IFT	Interfacial tension
KCl	Potassium chloride
KI	Potassium iodide

M	Molarity
m	meter
mg	Milligram
min	Minute
mL	Milliliter
mN/m	Milli-Newton per meter
mPa	Millipascal
mV	Millivolt
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
NCS	Normal corn starch
nm	Nanometer
OSA	Octenyl succinic anhydride
OS	Octenyl succinic
%OS	Weight percentage of OS groups in the OS starch
O/W	Oil-in-water
PS	Pea starch
psi	Pounds per square inch
r	Particle radius
RE	Reaction efficiency
RVA	Rapid Visco Analyser
rpm	Revolutions per minute
s	Second
SDS	Sodium dodecyl sulfate solution
T_c	Conclusion temperature
T_o	Onset temperature
T_p	Peak temperature
v	velocity (m/s)
v/v	Volume to volume
WCS	Waxy corn starch
W/O	Water-in-oil

wt%	Weight percentage
w/v	Weight to volume
w/w	Weight to weight
ξ	Zeta potential
ρ	Density (kg/m^3)
η	Viscosity (mPa/s)
µm	Micrometer
µL	Microliter

1. INTRODUCTION

1.1 Overview

Pulses include dried, edible seeds of *Leguminous* plants. Canada is the second largest producer of pulses globally (Hoover, Hughes, Chung, & Liu, 2010; Maaran, Hoover, Donner, & Liu, 2014). In recent years, an increasing volume of pulses is processed to produce value-added ingredients, such as pulse protein concentrate/isolate, to improve their commercial value (Asif, Rooney, Ali, & Riaz, 2013). However, pulse starch, as one of the major co-products from the processing, shows low application value in the industry due to the poor inherent functional properties. Therefore, modification of pulse starch is required in order to expand its industrial utilization. Various modification methods, including chemical, enzymatic, physical, genetic and combinations of these methods, have been employed for this purpose (Zia-ud-Din, Xiong, & Fei, 2017).

Octenyl succinic anhydride (OSA) modification is a chemical method that is commonly employed to modify starch in the food industry. This modification is unique as it introduces hydrophobic octenyl succinic (OS) groups onto starch molecules through esterification, which enables starch to function as an emulsifier to stabilize emulsion. Food and Drug Administration (FDA) has approved the food use of OS starch. The allowed level of OSA used in the preparation of food-grade starch is 3% based on the dry weight of starch (Rayner et al., 2014). With an overarching goal to expand the industrial use of pulse starch, this thesis research aimed to: (1) develop emulsifiers from a representative pulse starch – pea starch (PS) – by using OSA modification; and (2) determine the emulsifying properties of the generated OS PS and the stability of the prepared OS-PS emulsions.

In this study, PS isolated from starch-rich pea flour, along with commercial normal corn starch (NCS) and waxy corn starch (WCS) was modified with 1%, 3% and 5% OSA (w/w, db). After the modification, the degrees of substitution (DS), thermal properties, and pasting properties of the OS starches were determined, and the effects of DS on the gelatinization and pasting behaviors of OS starches were investigated. Oil-in-water (O/W) emulsions that contained 5 wt% of canola oil and 2 wt% of OS starches were prepared through high pressure homogenization. The resultant emulsions were characterized with respect to droplet-size distribution and zeta potential to evaluate the emulsifying properties of the OS starches. Emulsion stability against accelerated storage, 28-d storage (at 4°C), pH change from 2 to 7, the presence of sodium chloride at 0-1.0 M, as well as heating treatment (90°C for 30 min) was evaluated. Confocal laser scanning microscopy (CLSM) was performed in order to visualize the changes in the formed emulsions under the test conditions. Gum Arabic (GA) was included as the industry standard of emulsifier throughout the characterization of the emulsions.

The thesis study illustrated the possibility of utilizing OS PS as an emulsifier in food, cosmetic and pharmaceutical industries. The fundamental research has provided directions for additional studies to further enhance the emulsifying capability and emulsion stability of OS PS.

1.2 Objectives

(1) To modify PS, NCS and WCS with 1%, 3% and 5% OSA (w/w, db);

(2) To characterize the structures and physicochemical properties of the derived OS starches, including DS, thermal properties, and pasting properties;

(3) To examine the emulsifying ability of the OS starches in canola oil-in-water emulsions and the stability of the resultant emulsions upon accelerated storage and 28-d storage (at 4°C);

(4) To investigate the stability of the OS-starch emulsions under various environmental conditions, including pH 2-7, 0-1.0 M sodium chloride, and incubation at 90°C for 30 min.

1.3 Hypotheses

(1) Gelatinization temperatures and enthalpy changes of OS starches will decrease as the DS increases; the percentages of retrogradation of OS starches will reduce with an increasing DS;

(2) OSA modification will reduce the pasting temperatures and increased the peak viscosities of OS starches, and more obvious effect will be observed for a higher DS;

(3) OS starches will show the capability of lowering oil-water interfacial tension and forming O/W emulsion;

(4) Emulsifying properties of OS PS, OS NCS, and OS WCS and stability of the prepared emulsions will be improved by a higher DS; emulsions prepared with OS starches will exhibit good stability upon 28-d storage and under the conditions of pH, ionic strength, and thermal treatment;

(5) OS WCS will show the strongest capability of stabilizing emulsions under the test conditions, followed by OS NCS and OS PS; stability of OS-starch emulsions will be comparable to that of GA emulsion under the same test conditions.

2. LITERATURE SURVEY

2.1 Utilization of pulses in human food

Pulses are defined as dried, edible seeds of *Leguminous* plants that include approximately 750 genus and 16,000-19,000 species (Maaran et al., 2014; Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001). According to the report of Joshi and Rao (2017), Canada was the second largest producer of pulses in the world between 2011 and 2013. In terms of total growing area and production, Saskatchewan is the leading producer of pulses in the country, followed by Alberta and Manitoba. Dry peas, lentils and chickpeas are the main pulse crops that are grown in Saskatchewan (Tosh & Yada, 2010).

In many parts of the world, pulses are consumed as whole or split seeds, such as canned pulses. Recently, pulses have attracted growing interest from the food industry due to their desirable nutritional value (*e.g.*, high contents of dietary fiber, protein, and minerals), low caloric value, and low glycemic effect (Asif et al., 2013; Chibbar, Ambigaipalan, & Hoover, 2010; Singh, Sandhu, & Kaur, 2004). Pulses can also be processed into food ingredients of various forms, including flour, starch, hull fiber and protein concentrate/isolate. These ingredients possess desirable functional and nutritional properties and are widely used in bakery products, snacks, sports foods, meat products *etc.*, which can substantially increase the utilization of pulses in the food industry (Barbut, 1999).

2.2 Value-added fractionation of pulses using dry- and wet-milling method

The commercial value of pulses can be improved by fractionating them into the aforementioned functional food ingredients. Two processing methods are commonly employed to achieve this goal: dry milling and wet milling (Hoover et al., 2010).

Dry milling, which includes dehulling of pulse seeds, milling in a dry state, and subsequent air classification, is the most common fractionation method used in the pulse processing industry in Canada. During the processing, pulse seeds are firstly de-hulled and finely ground into flour using hammer milling, pin milling or impact milling. The flour is further separated into starch-rich and protein-rich flours based on the differences in their densities and particle sizes using air classification technology: the starch-rich flour is coarser and denser, while the protein-rich flour is finer and lighter (Boye, Zare, & Pletch, 2010). In the dry-milling process, the air classification step can be repeated several times to enhance the separation efficiency (Boye et al., 2010; Tyler, Youngs, & Sosulski, 1981). However, the purity of the starch-rich and protein-rich flours obtained from the dry-milling method is poor because of the incomplete separation of these two major components in air classification (Meuser, Pahne, & Möller, 1995).

In the wet-milling process, the de-hulled seeds are typically soaked in water for 3-14 h first, which is followed by a series of steps of milling, extraction of pulse protein under alkaline conditions, and recovery of insoluble starch (Hoover et al., 2010; Tiwari, Gowen, & McKenna, 2011). In the alkaline extraction step, the suspension containing the finely milled pulses is usually adjusted to pH 8-11 to solubilize the protein, and the water-insoluble starch is separated from the solubilized protein using filtration (Boye et al., 2010). The solubilized pulse protein is recovered from the filtrate through precipitation at the isoelectric point (pH 4-5), centrifugation and drying (Hoover et al., 2010; Paredes-López, Ordorica-Falomir, & Olivares-Vázquez, 1991). The filtered starch cake is suspended in water to adjust to pH 7, followed by additional washing, filtration and drying to obtain isolated pulse starch. In comparison with dry milling, wet milling can yield starch and protein of a greater purity.

Protein-rich flour and protein concentrate/isolate have suitable functional properties and high nutritional value (*i.e.*, as a good source of plant protein) and thus they are widely used in the food industry (Boye et al., 2010). In contrast, starch-rich flour and pulse starch, as main co-products from pulse processing, are underutilized due to the poor inherent properties of pulse starch. The pulse industry in Canada is exploring new approaches to enhancing the functional properties and nutritional value of pulse starch for increased industrial utilization (Ratnayake, Hoover, & Warkentin, 2002).

2.3 Structure of pulse starch granules

2.3.1 Granular feature and structure of amylose and amylopectin in pulse starch

Pulse starch granules mostly have an oval, kidney, spherical, and irregular shape with width of 5-55 μm and length of 5-70 μm (Fig. 2.1) (Chibbar et al., 2010; Li et al., 2019). The surface of pulse starch granules is usually smooth with no obvious fissures or pin holes (Chung et al., 2008; Ratnayake et al., 2001).

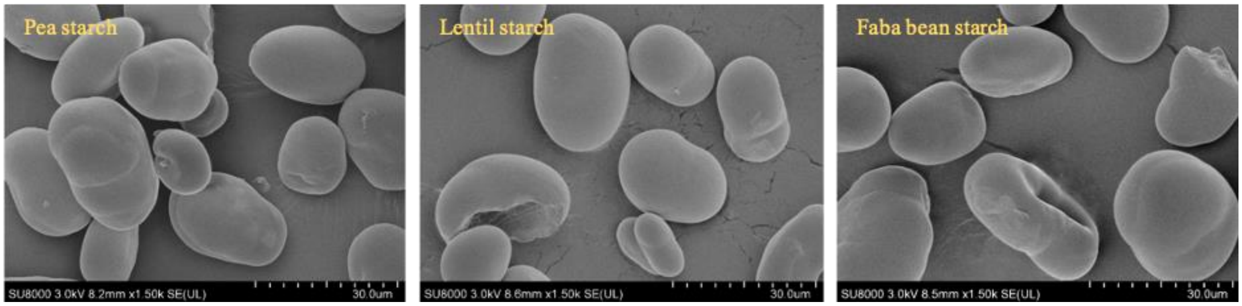


Fig. 2.1. Scanning electron microscopy images of pulse starches. Magnification is × 1500 [reproduced with permission from Li et al. (2019)].

Similar to starches of other botanical origins, amylose and amylopectin are the two major components in pulse starch. Amylose is an essentially linear molecule composed of D-glucopyranosyl units linked by α-(1→4) linkages, with a few α-(1→6) branch linkages (Fig. 2.2). Common methods that have been employed for the determination of amylose content in pulse starch include iodine potentiometric titration, iodine colorimetry, gel permeation chromatography and enzymatic amylose-amylopectin assay kit (Chibbar et al., 2010). Ratnayake et al. (2001) reported that amylose constitutes 24-88% of pulse starches. The amylose contents of some representative pulse starches are summarized in Table 2.1.

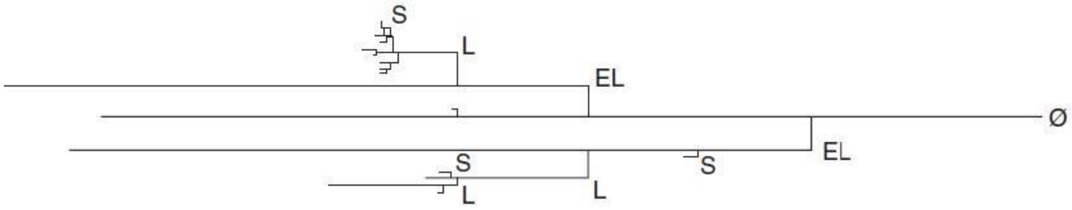


Fig. 2.2. Proposed structure of the branched amylose molecule comprising immature clusters. EL, extremely long; L, long; and S, short chains; Ø, reducing end [with permission from Takeda, Shitaozono, and Hizukuri (1990)].

Table 2.1 Amylose contents of representative pulse starches.

Starch	Amylose content (%)[a]
Smooth pea	32.5-33.0
Wrinkled pea	62.8-75.4
Chickpea	30.4-32.2
Faba bean	31.3-42.1
Lentil	29.0-45.5

[a]Hoover and Sosulski (1991)

Amylopectin is the primary component in most pulse starches and it has a higher degree of polymerization (DP; ~62,000 to 6,200,000) than amylose (~690 to 6340) (Aberle, Burchard, Vorwerg, & Radosta, 1994). The branch chains of amylopectin have been classified into A-, B- and C-chains (Fig. 2.3) by Hizukuri (1986). The shortest chains with DP between 6 and 12 are defined as A-chains, and A-chains do not carry any other branch chains. B-chains carry A-chains and other B-chains, and they can be further categorized into B1, B2, B3 and B4 chains based on the cluster numbers they spread through (Hizukuri, 1986). The C chain carries the sole reducing end of the amylopectin molecule (Gunja-Smith, Marshall, Mercier, Smith, & Whelan, 1970). The (1→4)-α-D-linked branch chains are connected by approximately 5% α-(1→6) branch linkages to form one amylopectin molecule.

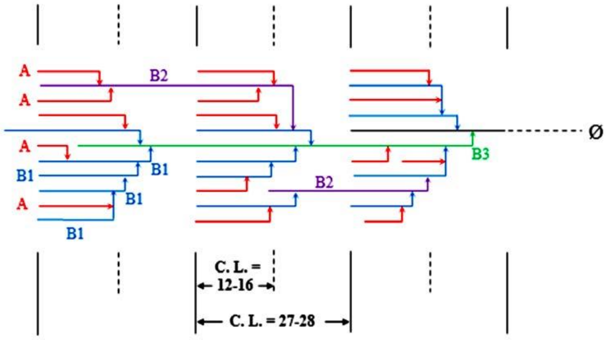


Fig. 2.3. A cluster model of amylopectin molecule with A, B1, B2, B3 and C chains. The one carrying the reducing end (Ø) is defined as C chain. C. L., chain length [with permission from Hizukuri (1986)].

2.3.2 Organization of starch molecules within granules

The crystalline structure of granules of common starches is attributed to the double helices formed between branch chains of amylopectin, while amylose is demonstrated to intersperse among amylopectin molecules (Fig. 2.4) (Hoover et al., 2010; Kasemsuwan & Jane, 1994). Starch granules from various plants show different polymorphic structures, which can be further classified into three patterns based on X-ray diffraction analysis: A-, B-, C-type (Bogracheva et al., 1999). Starches from most cereal grains display the A-type X-ray diffraction pattern, which has double-helical crystallites packed compactly in monoclinic unit cells with approximately 4 water molecules in one-unit cell (Fig. 2.5). In contrast, B-type is generally found in some tuber and root starches, and they have double-helical crystallites packed more loosely in hexagonal unit cells with approximately 36 water molecules in the center of one cell (Pérez, Baldwin, & Gallant, 2009). Pulse starches typically exhibit the C-type X-ray diffraction pattern, which is a mixture of A- and B-type polymorphs (Tiwari et al., 2011). A previous study on the semi-crystalline structure of pea starch carried out by Bogracheva, Morris, Ring, and Hedley (1998) revealed that B-type polymorphs were mainly located in the central part of the granules, while A-type polymorphs were mainly present in the peripheral region.

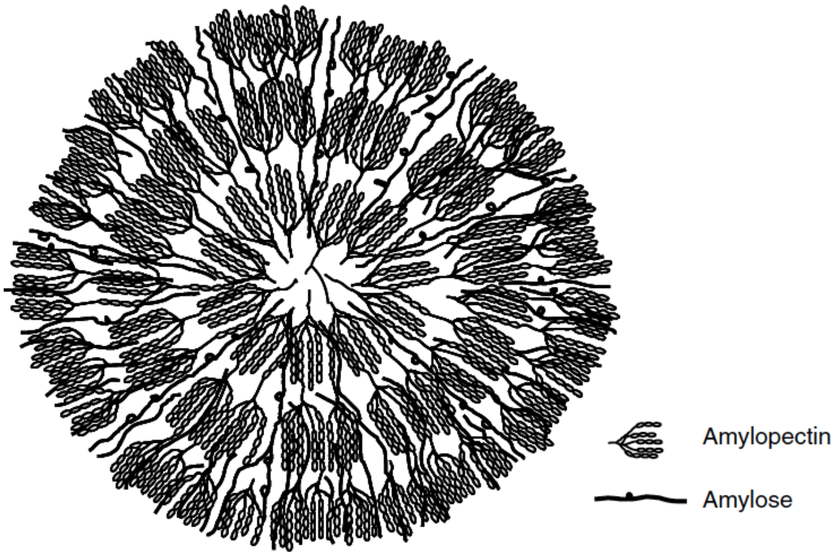


Fig. 2.4. Schematic of the organization of a starch granule [with permission from Jane (2009)].

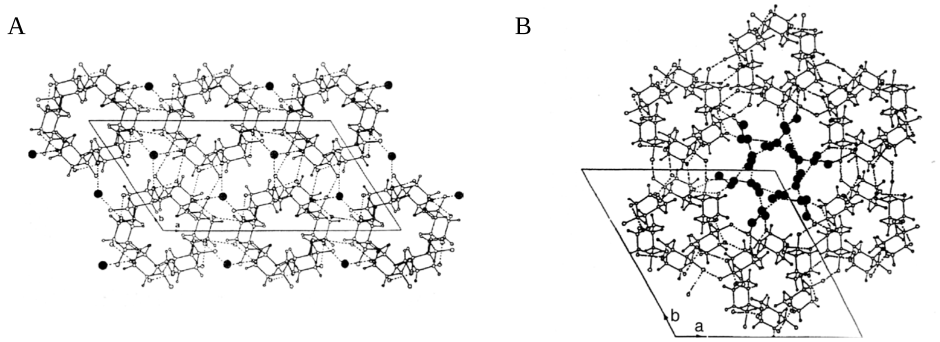


Fig. 2.5. (A) Structure of A starch; (B) Structure of B starch. Closed circles represented water molecules [with permission from Pérez et al. (2009)].

2.4 Octenyl succinic anhydride (OSA) modification of starch for enhancing functional properties

Starch is widely utilized in the food industry as an important ingredient. Starch can greatly influence the characteristics of the end products, including their texture, viscosity, stability *etc.* (Davies, 1995). However, the undesirable inherent properties of native starches, such as low stability during storage and poor resistance against shear, heat and low pH, limit their industrial applications (Jayakody & Hoover, 2008). Therefore, various modification methods, including chemical, enzymatic, physical, genetic and combinations of these methods, have been used to improve the properties of native starches for the utilization in a broader range of food products (Zia-ud-Din et al., 2017).

Chemical modification is most widely utilized to modify starch, in which chemical reagents are added to starch to introduce new functional groups onto starch molecules through the formation of new covalent bonds (Zia-ud-Din et al., 2017). Octenyl succinic anhydride modification is a common chemical method used for starch modification, and the modification procedure and characterization of OS starch will be discussed in this section.

2.4.1 Modification procedure of OS starch

Octenyl succinic anhydride (OSA) is a compound that can esterify with native starches and generate modified starches with strong amphiphilic interfacial activity. The esterification occurs between hydroxyl groups of starch and OS group as shown in Fig. 2.6. This reaction is usually carried out on native, granular starch in an aqueous medium at a weak alkaline pH ranging from 7.2 to 8.5 (Abiddin, Yusoff, & Ahmad, 2018). It has been shown that the substituted OS groups are heterogeneously distributed within a starch granule and among starch granules, and the reaction is preferentially to occur on the surface and the amorphous region of starch granules (Huang et al., 2010; Wetzel, Shi, & Reffner, 2010). Two parameters, degree of substitution (DS) and reaction efficiency (RE), are commonly used to evaluate the extent of the modification, and the determination of DS and RE will be introduced in Section 2.4.2.

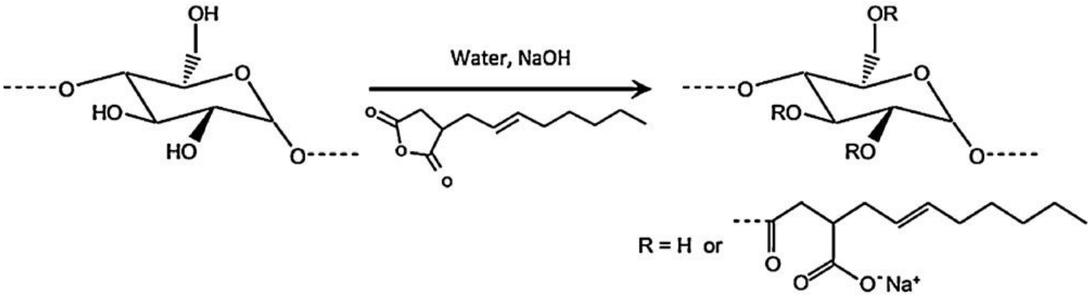


Fig. 2.6. Reaction of OSA with starch to develop of OS starch [with permission from Altuna, Herrera, and Foresti (2018)].

Besides conducting the reaction in alkaline aqueous medium, several additional treatments can be applied to improve the reaction efficiency, such as mechanical treatment, ultrasonic-assisted treatment, hydrothermal and enzyme pre-treatment (Altuna et al., 2018). According to a previous study of Wang, He, Fu, Luo, and Huang (2015), the reaction efficiency increased by 8% when the sample was subjected to high-speed stirring (10,000 rpm) compared to a normal condition (500 rpm). The authors attributed the results to the disruption of starch granule surface and the reduction of OSA droplet size, which enhanced the penetration of OSA into the interior of starch granules. The results reported by Chen, He, and Huang (2014) showed that hydrothermal treatment prior to OSA modification of corn starch increased the reaction efficiency from 72.2 to 81.8%. The improvement in RE was explained by the increasing porosity of the pretreated granules, which enhanced the granule accessibility and eventually led to easier migration of OSA droplets to the inside of starch granules.

After the modification with OSA, the originally hydrophilic starch possesses an amphiphilic feature due to the addition of hydrophobic OS groups (Fig. 2.7). OS starches can be dispersed in water by heating to destroy the granular form, and the dispersed amphiphilic OS starch molecules can act as an emulsifier. Thus, OS starch shows promise for diverse industrial applications, especially in emulsifications and encapsulations. FDA has approved the use of OSA modified starch in foods. The allowed level of OSA used in the modification of starch for food use is 3% on the dry weight of starch (Rayner et al., 2014).

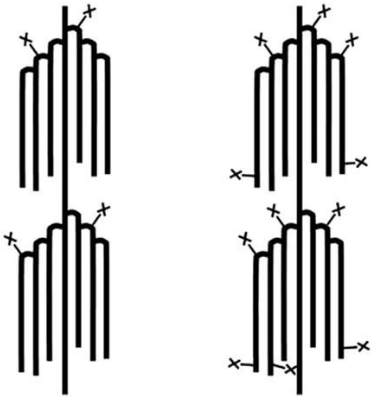


Fig. 2.7. Proposed model structures for octenyl succinic starches with degree of substitution (DS) of 0.018 (left) and 0.092 (right) [with permission from Bai, Kaufman, Wilson, and Shi (2014)].

2.4.2 Determination of degree of substitution in OS starch

Degree of substitution (DS) is used to describe the extent of OSA modification, which is defined as the average number of OS groups attached to one glucose unit in starch. The percentage of the modification attained is expressed as reaction efficiency (RE), which is calculated by dividing the OS weight percentage in OS starch over the %OSA added to the reaction system (Altuna et al., 2018).

Three methods are commonly used to measure the DS of OS starch, which include titration method, Fourier transform infrared spectroscopy and NMR spectroscopy. Most previous studies used titration method to determine the DS in OSA modified starch, and two types of procedures have been reported in previous literature (Bhosale & Singhal, 2006; Lopez-Silva, Bello-Perez, Castillo-Rodriguez, Agama-Acevedo, & Alvarez-Ramirez, 2020; Zhang, Mei, Chen, & Chen, 2017). The first method is based on the saponification reaction occurring between OS-starch and alkaline, and the excess alkali is back titrated with acid. Generally, alkaline solution is added to suspend the modified starch, which leads to saponification reaction of OS starch. Hydrochloric acid solution is then added to titrate and neutralize excess alkaline in the system with the presence of an indicator (*e.g.*, phenolphthalein and methyl orange). In the other method, the OS starch is suspended in hydrochloric acid or isopropanol solution, filtered and washed with distilled water to remove all the Cl^{-} ions, and then resuspended in distilled water. The suspension is cooked in a boiling water bath and then titrated with sodium hydroxide. Native starch is required to be titrated as blank in both methods for accurate calculation of DS and RE.

2.5 Physicochemical properties of OS starch

OSA modification introduces hydrophobic OS groups to the hydrophilic starch molecules, which enables the modified starch to locate at interfaces and act as an emulsifying agent in an emulsion system. Meanwhile, the physicochemical properties of OS starch are also altered due to the addition of OS groups. Generally, OS starch shows lower gelatinization temperatures, a higher peak viscosity and less retrogradation tendency when compared with its native form.

2.5.1 Gelatinization property

Gelatinization refers to the thermal transition of starch from a semi-crystalline structure to an amorphous state upon heating in the presence of excess water. Gelatinization properties of starch are usually determined using differential scanning calorimetry (DSC) (Hoover et al., 2010). Briefly, starch is heated in DSC with excess water and the heat flow as a function of temperature during the scanning is plotted. The starch gelatinization temperatures are calculated from the curve, and the enthalpy change (ΔH) is calculated from the peak area under the curve as shown in Fig. A1 in the Appendices. Previous research has shown that OS starches displayed much lower gelatinization temperatures and ΔH than their native counterparts and a greater extent of decrease was observed with a higher DS (Ai, Nelson, Birt, & Jane, 2013; Han & BeMiller, 2007). Table 2.2 summarized gelatinization temperature and ΔH results of OS starches and their native counterparts obtained from some pervious papers. Bhosale and Singhal (2007) attributed the reduced gelatinization temperatures of OS starch to the disruption of starch crystalline structure by the repulsion between OS groups.

Table 2.2 Gelatinization properties and %retrogradation of native and OS starches.

Starch	Gelatinization of starch				%retrogradation	Reference
	Onset temperature (ºC)	Peak temperature (ºC)	Conclusion temperature (ºC)	ΔH (J/g)		
Corn starch	65.5	70.3	74.8	12.9	-	(Ai et al., 2013)
OS (3%) corn starch	62.6	68.9	74.2	12.4	-	
OS (10%) corn starch	60.7	69.4	77.2	11.6	-	
Waxy corn starch	69.3	74.9	87.9	19.2	-	(Han & BeMiller, 2007)
OS (3%) waxy corn starch	67.5	72.8	82.3	15.4	-	
Rice starch	73.5	77.8	83.5	15.1	39.5%	(Thirathumthavorn & Charoenrein, 2006)
OS rice starch	68.7	73.9	80.6	12.2	28.5%	
Tapioca starch	63.7	68.4	79.8	14.3	23.2%	
OS tapioca starch	59.7	65.6	79.7	13.7	3.5%	
Sago starch	70.1	75.3	80.3	12.3	54.8%	(Abiddin et al., 2018)
OS (5%) sago starch	67.6	73.8	79.8	10.3	43.9%	

2.5.2 Swelling power and water solubility

When heating starch sample in excess water, the crystalline structure of native starch is destroyed and the hydroxyl groups of amylose and amylopectin are exposed to form hydrogen bonds with water molecules, leading to an increase in granule swelling and solubility (Ratnayake et al., 2002). OSA modification has been demonstrated to enhance the swelling power of starch. OS waxy corn, rice and wheat starch displayed a markedly higher swelling power compared to their respective native starches, and the enhancing effect increased with a larger DS (Bhosale & Singhal, 2007).

2.5.3 Pasting property

Pasting property is defined as the process of viscosity development when starch is further heated and stirred after gelatinization in water. During this process, starch granules continue to swell and some amylose and small amylopectin molecules leach out from the granules to provide the viscosity (Tester & Morrison, 1990). Rapid Visco Analyser (RVA) and Amylograph are most commonly deployed to determine the pasting properties of starch. Generally, starch slurry is heated and cooled with a programmed temperature profile under constant stirring, and the viscosity of the starch sample is determined using an equipped sensor in the whole process. The pasting profile of the starch is constructed by plotting the viscosity as a function of time. Important pasting parameters are determined from the pasting profile as shown in Fig. A2 in the Appendices. Pasting temperature is defined as the temperature at the onset of viscosity development. In the pasting curve, the viscosity of starch keeps increasing until the majority of granules reach the maximum swelling, which is defined as the peak viscosity. After reaching the peak viscosity, the viscosity starts to decrease due to the disruption of swollen starch granules, and the minimum viscosity is defined as the holding strength, while the breakdown viscosity is defined as the difference between the peak viscosity and the holding strength. In the last stage of the test, the starch paste is cooled to a certain temperature, which leads to an increase in the viscosity. At the end point, the viscosity is recorded as the final viscosity. The setback viscosity is defined as the difference between the final viscosity and the holding strength (Wani et al., 2012).

OS starches prepared from various botanical sources generally exhibit a lower pasting temperature and higher peak viscosity than the corresponding native starches as shown in Fig. 2.8 (Bao, Xing, Phillips, & Corke, 2003; Han & BeMiller, 2007; Zhu, Xie, Song, & Ren, 2011). The OS groups disrupt the structure of starch granules and lead to easier water penetration of OS starch, which results in granular swelling at lower temperature than the native starch (Bello-Flores, Nuñez-Santiago, San Martín-Gonzalez, BeMiller, & Bello-Pérez, 2014). The incorporation of OS groups cause repulsion in the starch granules, which leads to greater extent of granular swelling and subsequently increase the pasting viscosities (Zhu et al., 2011).

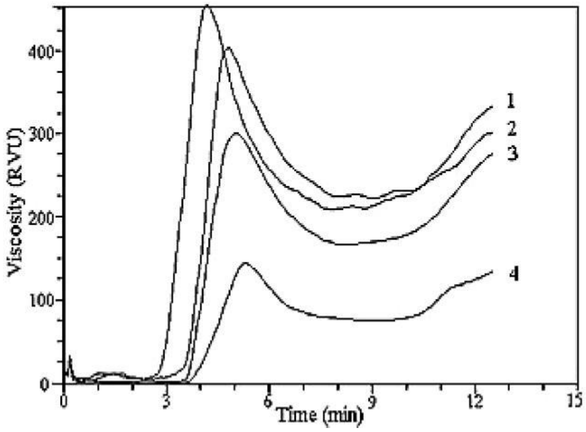


Fig. 2.8. Pasting curves of native and OS starches. (1) DS=0.031; (2) DS=0.025; (3) DS=0.018; (4) native starch [with permission from Song, He, Ruan, and Chen (2006)].

2.5.4 Retrogradation

When gelatinized starch is stored at a relatively low temperature (*e.g.*, 4-25°C), adjacent starch chains will form double helices to partially restore the crystalline structure of starch, which leads to a return of starch from a solvated, dispersed, and amorphous state to an insoluble, aggregated and crystalline condition. This process is known as starch retrogradation (Ratnayake et al., 2002). Starch retrogradation is associated with the syneresis of starch gel and the staling of bread during storage (Hoover et al., 2010). Retrogradation of starch can be determined using DSC and X-ray diffraction technique. OS starch has been illustrated to display a less tendency to retrograde than native starch in previous studies (Table 2.2, %retrogradation). The phenomenon is attributed to the prevention of re-association between starch molecules by the introduction of OS groups (Song, Zhao, Li, Fu, & Dong, 2013; Thirathumthavorn & Charoenrein, 2006).

2.5.5 Gel formation

Starch gel is formed after gelatinized and swollen starch granules fill the mold and form a network to hold water through the interactions between amylose and amylopectin. The rheological properties of starch gel can be characterized using a texture analyzer or a rheometer. Previous research has reported that OS corn starch and OS amaranth starch showed strong shear-thinning behavior where increasing shear rates resulted in decrease in viscosity of the OS starch paste. The phenomenon was explained by the following reasons: (1) higher shear rate disentangles the arrangement of starch long chains and helps to overcome the intermolecular resistance to flow; (2) increasing shear rate shears away the solvated particles in the OS starch dispersion causing reduction in effective particle size and thus decrease the apparent viscosity (Bhandari, Singhal, & Kale, 2002). Both storage modulus (G') and loss modulus (G") of OS starch paste were reported to increase with a higher degree of OSA modification (Park, Chung, & Yoo, 2004).

2.6 Emulsifying properties of octenyl succinic anhydride modified starch and stability of the resultant emulsions

2.6.1 Emulsion and emulsifiers

Emulsion is a system that includes two immiscible liquid phases (*e.g.*, oil and water): the one forming droplets is the dispersed phase and the other one is the continuous phase. Two common types of emulsion are oil-in-water (O/W) emulsion (Fig. 2.9. A), in which oil droplets are dispersed in the aqueous phase (*e.g.*, milk, cream and salad dressing), and water-in-oil (W/O) emulsion (Fig. 2.9. B), in which water droplets are dispersed in the oil phase (*e.g.*, butter, spreads and margarine) (Berton-Carabin & Schroën, 2015). Rapid phase separation usually occurs to the emulsion system formed with pure oil and water because the droplets tend to collide together and merge into one droplet to minimize the contact area between both phases, which subsequently results in phase separation. The separation is driven by the fact that the contact between oil and water is unfavorable in thermodynamic aspect, therefore, emulsion is considered as a thermodynamically unstable system (Israelachvili & Wennerstroem, 1992; McClements, 2015).

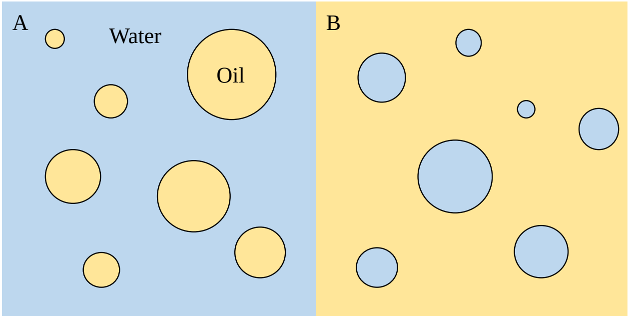


Fig. 2.9. Schematic diagram of (A) oil-in-water (O/W) emulsion (B) and water-in-oil (W/O) emulsion.

Emulsion stabilizers can be used to improve the stability of emulsion and delay the phase separation, which are classified into two types: thickening agents and emulsifiers. Thickening agents (*e.g.*, xanthan gum) can increase the viscosity of the continuous phase of emulsion and hence delay the creaming of droplets (Dickinson, 2009). Emulsifiers are surface-active molecules that contain both polar and nonpolar groups. These amphiphilic molecules can absorb on the oil-water interface and form a membrane on the surface of the droplets that can prevent them from aggregation (McClements, 2015). The film produced by emulsifiers provides repulsive droplet-droplet interaction that contributes to the emulsion stability, which includes steric repulsion (due to overlapping of emulsifier on the interface of two droplets) and electrostatic repulsion (emulsifiers with charge) as shown in Fig. 2.10. The driving force for phase separation still presents in all food emulsion systems, and the emulsifiers keep the systems stable for a reasonable shelf life before obvious physical destabilization occurs. Therefore, the emulsion is a kinetic stable or metastable system (Berton-Carabin & Schroën, 2015; McClements, 2015).

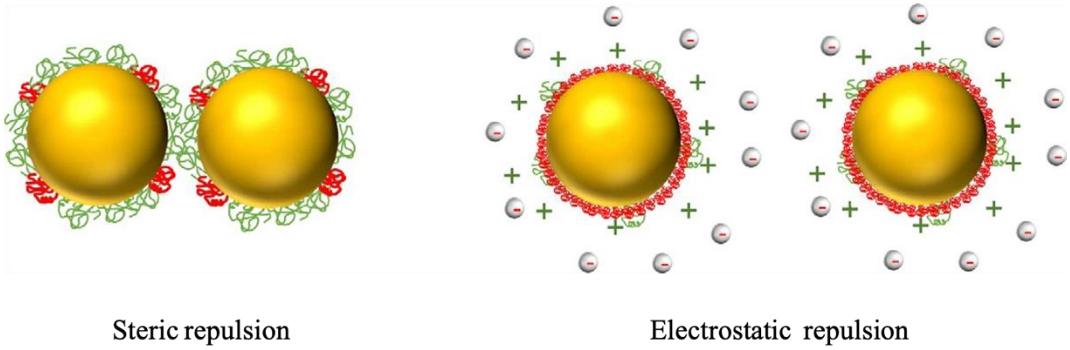


Fig. 2.10. Schematic diagram of steric repulsion and electrostatic repulsion between emulsion droplets [reproduced with permission from McClements and Jafari (2018)].

There are mainly two categories of emulsifiers: small-molecule surfactants and amphiphilic biopolymers. Small molecule surfactants that commonly use in food industry are chemically synthesized molecules using various raw materials, such as glycerol, fats, organic acids and sugars. Those surfactants include Tweens, Spans, CITREM and DATEM (Kralova & Sjöblom, 2009). Typically, small-molecule surfactant contains a hydrophilic head (polar) and a hydrophobic tail (non-polar). Some naturally found surfactants, for example, saponins, show ellipsoid structures with a polar side and a non-polar side (Fig. 2.11) (Berton-Carabin & Schroën, 2015; Pagureva et al., 2016).

Protein is a representative category of amphiphilic biopolymers that can be used as an emulsifier. Some commonly used food-grade protein emulsifiers include whey proteins, caseins, egg proteins, soy proteins and pea proteins (Lam & Nickerson, 2013). In order to be good emulsifiers, an appropriate ratio of the hydrophilic and hydrophobic groups on protein surface is required. Protein emulsifiers have two types of structures, random coil structure (*e.g.*, β-lactoglobulin) and globular structure (*e.g.*, caseins), which influence the stability of emulsions formed by different proteins (Fig. 2.11) (McClements & Jafari, 2018). The interfacial layer produced by proteins are generally thin and electrically charged, and thus the electrostatic repulsion plays a major role in stabilizing protein-based emulsions. As a consequence, the stability of protein-based emulsions is largely impacted by pH and ionic strength. The emulsion tends to flocculate when pH is close to the isoelectric point of protein and when ionic strength is higher than a certain level (McClements, 2004). Additionally, emulsion formed with globular proteins are sensitive to change of temperature due to the reactive nonpolar and sulfhydryl groups of globular proteins will be exposed at critical temperature values. The attractive interactions among emulsion droplets increase by these groups, resulting in flocculation of droplets (McClements, 2015).

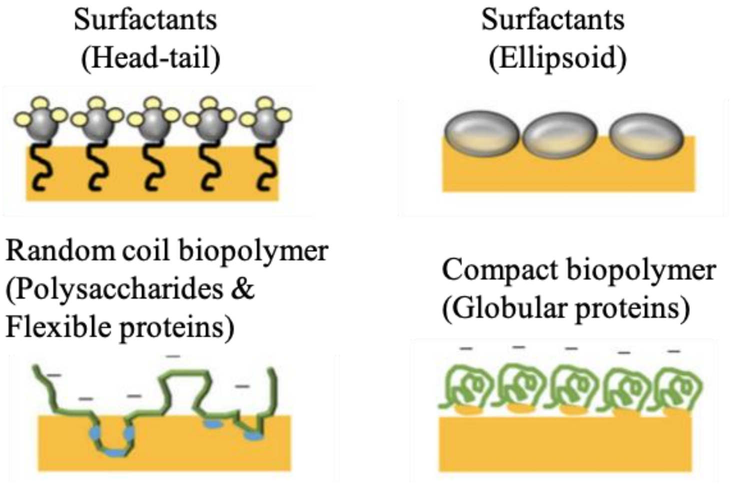


Fig. 2.11. Schematic diagram of emulsifiers that can be used to stabilize emulsions [reproduced with permission from McClements and Jafari (2018)].

Surface-active polysaccharides can act as emulsifiers, for instance, gum Arabic and modified starch (Dickinson, 2009). Gum Arabic is obtained from the natural exudate of Acacia Senegal that consists of anionic arabinogalactan, arabinogalactan protein and glycoprotein. The hydrophilic polysaccharide chains are attached to the hydrophobic polypeptide backbones. After adsorbing to the oil-water interface, the polysaccharide extends to the water phase while the polypeptide protrudes to the oil phase (Fig. 2.11) (McClements & Gumus, 2016; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). Gum Arabic forms a relatively thick coating on the surface of oil droplet, which provides strong steric repulsion among the droplets. Therefore, the steric repulsion is believed to mainly prevent droplet aggregation, and the electrostatic repulsion makes some contribution in stabilizing emulsion as well (McClements, 2015). Native starches are hydrophilic molecules that are not suitable for using as emulsifiers. However, non-polar hydrocarbon chains can be covalently bonded to starch chains through chemical modification method to make them into effective emulsifiers (*e.g.*, OSA modification introduced in Section 2.4). Similar to gum Arabic, the hydrophobic groups of modified starch anchor in oil phase, whereas the hydrophilic starch chains extend to water phase and form a layer around the oil droplet (Fig. 2.11). The steric repulsion produced by starch chains is the dominant mechanism in stabilizing emulsion. Other polysaccharide-based emulsifiers include modified celluloses (*e.g.*, methyl cellulose), pectin and chitosan (McClements, 2015; McClements & Jafari, 2018).

2.6.2 Destabilization of emulsion

Emulsions are thermodynamically unstable systems that face many different destabilization issues during production, storage and end use, which includes physical destabilization, chemical degradation and microbial spoilage (Berton-Carabin & Schroën, 2015). In this research, only physical destabilization is considered. Physical destabilization includes gravitational separation, flocculation, coalescence, Ostwald ripening, and phase inversion (Fig. 2.12) (Israelachvili, 2015).

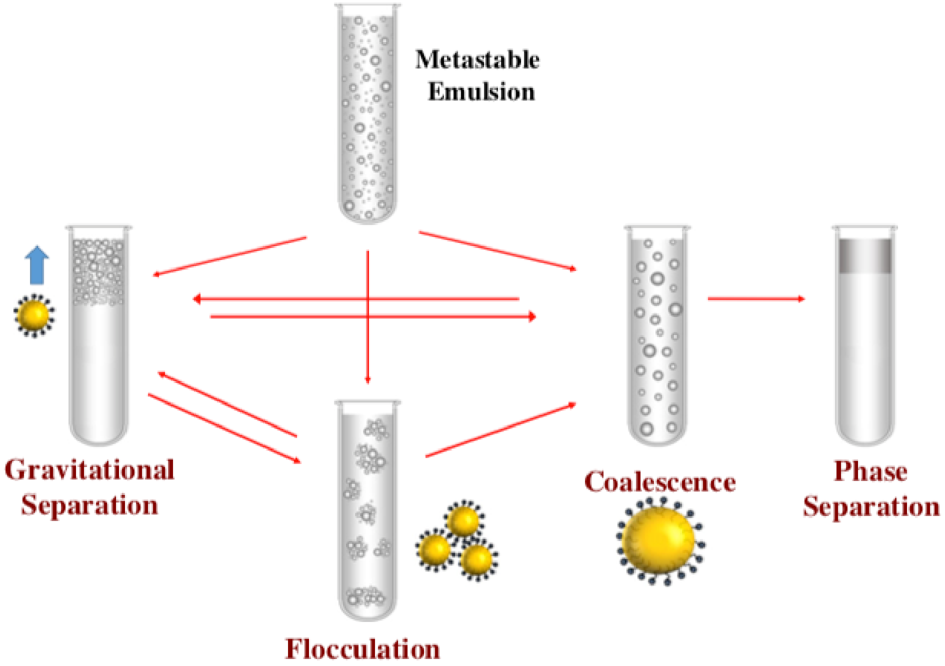


Fig. 2.12. Schematic diagram of physical destabilization processes in O/W emulsions, including gravitation separation, flocculation, coalescence, and phase separation [with permission from McClements and Gumus (2016)].

Gravitational separation is a common destabilization phenomenon that is caused by the difference in the density between the continues and dispersed phases. There are two types of gravitational separation: creaming and sedimentation. Creaming refers to the upward movement of oil droplets in O/W emulsion and the formation of cream layer due to the density of most edible oils are lower than that of water, whereas sedimentation refers to the downward movement of water droplets in W/O emulsion and the formation of aqueous layer (McClements, 2015). The gravitational separation of emulsion can be described using Stokes law (Hiemenz & Hiemenz, 1986):

$$v = \frac{2gr^2(\rho_2 - \rho_1)}{9\eta_1} \qquad (2.1)$$

Where v is the rate of gravitational separation (m/s) [the sign of v indicates the droplet moves upward (+) or downward (−)]; g is the gravitational acceleration (m/s^2); r is the radius of droplet (m); ρ_1 and ρ_2 are the densities of dispersed and continuous phases, respectively (kg/m^3); and η_1 is the viscosity of continuous phase (mPa/s). The equation suggests that gravitational separation rate positively correlates with droplet size and density difference between two phases, and negatively correlates with viscosity of continuous phase. Gravitational separation has an adverse effect on the quality of emulsion products, and thus this phenomenon needs to be retarded. Several ways can be conducted to control the gravitational separation based on Stokes law: 1. reducing the density difference by adding "weighting agents" to the oil phase; 2. decreasing the droplet size (*e.g.*, homogenization of raw milk); 3. increasing the viscosity of continuous phase using thickening agents (*e.g.*, xanthan gum) (Berton-Carabin & Schroën, 2015; McClements, 2015).

The phenomenon that two or more droplets aggregate together but maintain individual integrity is called flocculation, and the phenomenon may be reversible. Flocculation tends to occur when the attractive forces between droplets are stronger than the repulsive forces. The attractive forces include van der Waals, hydrophobic and depletion interaction while the repulsive forces include steric and electrostatic repulsion (Fig. 2.10) (Berton-Carabin & Schroën, 2015). Bridging flocculation and depletion flocculation are two phenomena that lead to droplet flocculation as well. Bridging flocculation typically occurs in emulsion stabilized by biopolymer when there is limited amount of emulsifier to fully cover the oil-water interface. Depletion flocculation occurs when there are excess emulsifiers existing in the continuous phase of emulsion. When two droplets get close to each other, the non-absorbed emulsifiers will be squeezed out from the narrow region between the droplets to the surrounding region that rich with emulsifiers. Therefore, the osmotic gradient difference between the two regions causes an increment in attractive force, leading to droplet flocculation (Dickinson, 2009; McClements, 2015). The effects of flocculation on the physical properties of emulsions are diverse. Flocculation may accelerate the rate of gravitational separation due to the increase in the effective droplet size. The viscosity of emulsion also increase due to flocculation increases the effective fraction of dispersed phase, which is undesirable in products that require low viscosity (Berton-Carabin & Schroën, 2015).

Coalescence is defined as two or more droplets aggregate together and merge into a larger droplet. There are three steps involved in coalescence: droplet deformation, film thinning and film rupturing. When emulsion droplets approach each other, the droplets start to deform. The interfacial film became flattened when the external forces applying to the droplets are stronger than the internal forces that keep the spherical shape of the droplets. The thickness of the film is thinning during the process and eventually ruptures, and hence the dispersed material in these droplets merges (Walstra, 2002). Coalescence is a thermodynamically preferable process as it decreases the total interfacial area, and it is irreversible. In emulsions, coalescence eventually results in phase separation, for instance, oil layer on the top of O/W emulsion and accumulation of water at the bottom of W/O emulsion (Berton-Carabin & Schroën, 2015; McClements, 2015).

Ostwald ripening is a process that large droplets grow, and small droplets shrink over time because of the mass transport from small droplets to large droplets through the continuous phase. This phenomenon typically occurs in O/W emulsions that consist water-soluble oils, including flavor oils, essential oils and short chain triglycerides (McClements & Gumus, 2016). The solubility of the dispersed material in a droplet increases with decreasing droplet size. Therefore, the concentration of dispersed material around small droplets is higher than that around the large droplets. As a result, the dispersed material molecules diffuse from small droplets to large ones and cause destabilization of emulsion. In most food emulsions, Ostwald ripening is negligible due to the low mutual solubilities of water and triacylglycerols (McClements, 2015).

Emulsions may undergo phase inversion in some cases, for example, the manufacture of butter. It is a process that the initial O/W emulsion turns to W/O emulsion and vice versa. Phase inversion can be induced by the alteration in emulsion composition or environmental conditions, including volume fraction of dispersed phase, type and concentration of emulsifier, temperature, ionic strength and et al (Berton-Carabin & Schroën, 2015; McClements, 2015).

2.6.3 Emulsifying properties of OS starch

The introduction of hydrophobic and bulky OS groups renders OS starch amphiphilic, which allows it to function as a surface-active agent and absorb at the air-water or oil-water interface (Song et al., 2013). In the emulsion forming process, OS starch suspension is firstly heated to fully gelatinize the starch in order to obtain a starch dispersion. Oil is added to the dispersion and the mixture is homogenized at a high pressure to generate a fine emulsion. During homogenization, OS-starch molecules migrate from aqueous phase to oil-water interface and absorb at the surface of droplet. The hydrophobic OS groups locate in the dispersed phase, whereas the hydrophilic starch chains extend to the continuous phase and formed a layer on oil droplet. The steric repulsion provided by starch layer prevents the emulsion from gravitational separation during storage (Fig. 2.13) (Lin, Liang, Zhong, Ye, & Singh, 2018b; Xu, Wang, Fu, Huang, & Zhang, 2018; Zhang et al., 2018).

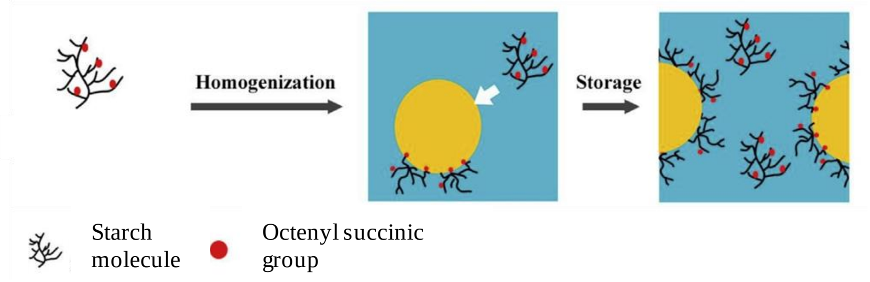


Fig. 2.13. Schematic illustration of the emulsifying and stability mechanism of OS starch [reproduced with permission from Zhao et al. (2018)].

It has been demonstrated that OS starch possesses a strong capacity to reduce the interfacial tension between two phases in emulsion. The study of Shogren and Biresaw (2007) showed that OS waxy corn starch could lower the interfacial tension of air and water from 50 to 10 mN/m, which was significantly lower than other starch derivatives at the same emulsifier concentration, such as waxy corn starch acetate (18 mN/m) and high-amylose corn starch acetate (15 mN/m). Emulsions stabilized by OS starch displayed droplets of small diameters. The average diameter of droplets in fresh emulsion stabilized by OS waxy corn starch at an emulsifier to oil mass ratio of 1:9 was 1.0 µm, similar to that of the droplets in the emulsion prepared with gum Arabic as reported by Xu et al. (2018).

2.6.4 Stability of OS-starch-based emulsions

The emulsions prepared with OSA modified starch display desirable stability during storage. Reiner, Reineccius, and Peppard (2010) reported that the mean droplet diameters of orange terpene-in-water emulsions stabilized by three commercial OS starches ranged from 0.72 to 1.64 µm. The mean diameters and size distributions of droplets in the three emulsions did not change significantly during 28-d storage. Olive oil-water emulsion prepared with OSA modified waxy corn starch did not show any oil separation after being stored at room temperature for 20 days, whereas the native starch failed to form an emulsion (Liu et al., 2008). Additionally, the study of Dokić, Krstonošić, and Nikolić (2012) showed that the droplet sizes of emulsions dropped from 5.7 to 4.2 µm with an increase of OS waxy corn starch concentration (8-16 wt%) and that the emulsion stabilized by the highest concentration of OS starch (16 wt%) also exhibited the lowest creaming index (0.45) during the storage of 16 days. These results indicated that the use of a higher concentration of OS starch in the emulsion system led to the formation of a more stable emulsion with smaller droplets.

When used as an emulsifier for food applications, OS starch has the advantages of being almost colorless and tasteless. Moreover, the stability of emulsion created with OS starch is largely independent of pH and ionic strength in the system. This is associated with the fact that the dominant stabilization mechanism of OS starch as an emulsifier is steric repulsion (Erni et al., 2007). In the paper published by Charoen et al. (2011), the commercial OSA modified starch was able to generate emulsions with droplets smaller than those prepared with whey protein isolate and gum Arabic even at higher concentrations. In addition, the changes in pH, ionic strength and temperature within the tested ranges had negligible impact on the mean droplet diameter and ζ-potential of OS starch stabilized emulsions. The emulsion prepared with a commercial OS starch also exhibited a smaller droplet size than that stabilized by whey protein isolate under various environmental conditions in the study conducted by Chanamai and McClements (2002).OS waxy corn starch has also been reported to be able to stabilize orange oil-in-water emulsion under a broad range of pH and NaCl concentration, without significantly changing the droplet sizes (Xu et al., 2018).

2.6.5 Applications of OS starch stabilized emulsions in foods and beverages

Because of the approved food-grade status, low price and desirable emulsion stabilizing capacity, there is growing interest in utilizing OS starch as a promising replacer for other food-grade emulsifiers, such as gum Arabic and proteins (Lin et al., 2018b). Studies have focused on using OSA modified starches to stabilize emulsion-based delivery systems to improve the bioavailability of target bioactive substances, such as β-carotene and curcumin (Lin et al., 2018b; Pan et al., 2019). Other applications of OS starch as emulsifiers in food products include carbonated beverages, juice drinks, energy drinks, alcoholic beverages, salad dressings and creams (Altuna et al., 2018).

2.7 Utilization of pulse starch for the preparation of OS starch

To the best of our knowledge, pulse starches have not been utilized to prepare any commercial OS starch products. It is unclear how OSA modification would influence the structure and functional properties of pulse starches. Also, there is a lack of understanding of how the unique structure and functional properties (*e.g.*, relatively high amylose contents, low gelatinization temperatures, and low pasting viscosities) of native pulse starches would affect the emulsifying properties and emulsion stability of their OS derivatives. In this thesis research, we aimed to modify pea starch – a representative pulse starch – with OSA and comprehensively examine the structure and functional properties of the resultant OS pea starch, particularly its emulsifying properties. Commercial normal and waxy corn starches were included for comparison.

3. MATERIALS AND METHODS

3.1 Materials

Pea starch of 94.8% purity was isolated from starch-rich pea flour (Starlite, Parrheim Foods, Saskatoon, SK, Canada) following the method of Li et al. (2019). Normal corn starch (Gel™ 03420) and waxy corn starch (Gel™ 04230) were kindly provided by Cargill Inc. (Minneapolis, MN, U.S.A.). Gum Arabic (TIC Pretested®) was donated by TIC GUMS Inc. (Belcamp, MD, U.S.A.). 2-Octen-1-ylsuccinic anhydride (OSA), Nile Red and Nile Blue A were purchased from Sigma-Aldrich Co. (Oakville, ON, Canada). Canola oil was purchased from a local grocery store. All the other chemicals used in this study were reagent grade and purchased from Fisher Scientific Company (Ottawa, ON, Canada) or Sigma-Aldrich Canada Co. (Oakville, ON, Canada).

The chemical compositions of the three starches are presented in Table A1 in the Appendices. The starch contents of the starches ranged from 94.8% to 97.5% (dry basis), with only 0.1%-0.3% protein and 0.03%-0.15% ash, which indicated the high purity of the starch samples in this study. Therefore, the impact of other components, particularly protein, on the emulsifying properties of the derived OS starches was negligible.

3.2 Methods

3.2.1 Amylose content of native starch

Amylose content of native starch was determined to evaluate the effect of starch structure on emulsifying property of corresponding OS starch and stability of resultant emulsion. Iodine affinity of pea starch (PS), normal corn starch (NCS) and waxy corn starch (WCS) was quantified by potentiometric titration using an auto-titrator equipped with Pt-ring and calomel reference electrodes. Starch sample was dispersed in dimethyl sulfoxide (DMSO; 90% solution in water, v/v) by heating in a boiling water bath, which was followed by precipitation with five volumes of anhydrous ethanol and centrifugation at 3000 *g* for 15 min. The supernatant was carefully decanted to remove the dissolved endogenous lipids (Li, Jiang, Campbell, Blanco, & Jane, 2008). The starch sediment was dried at 105°C in a convection oven overnight. The iodine affinity of the defatted, dried starch (100.0 mg, db) was determined by titrating with a solution containing 50 mM KI, 50 mM KCl and 0.8 mM I_2. The obtained value was converted to apparent amylose content afterdividing by a conversion factor of 0.2 (the iodine affinity of purified amylose) (Takeda et al., 1990). The determination of each starch was done in triplicate.

3.2.2 Octenyl succinic anhydride modification of starch

The octenyl succinic anhydride (OSA) modification of PS, NCS and WCS was carried out in accordance with the method of Ai et al. (2013) with minor changes. The starch sample (35.0 g, db) was suspended in distilled water (35%, w/w) under stirring condition at ~450 rpm. Sodium hydroxide (3%, w/v) was added to the suspension to adjust the pH to 8.0 and the temperature was kept at 35°C. OSA (1%, 3% and 5%, w/w, db) was added drop by drop into the mixture while maintaining the pH at 8.0 and the temperature at 35°C. At the end of the modification, the pH of the starch suspension became stable at 8.0. The modification took from 2 to 5 h varying from starch type and OSA level. The suspension was then adjusted to pH 6.5 by adding 1.0 M HCl. The modified starch was recovered through centrifugation at 5000 *g* for 10 min, washed twice with distilled water (100 mL) and twice with absolute ethanol (100 mL), and then dried at 37°C in a convection oven overnight. The dried starch was ground using a mortar and pestle to fine powder for further analyses. The starch underwent the same modification process without the addition of OSA was used as the control. The OS starches were named as "OS (% used for modification) abbr. of starch" [*e.g.*, OS (1%) PS]. The control starches were named as PS-C, NCS-C and WCS-C.

3.2.3 Degree of substitution and reaction efficiency of OS starch

A titration method was applied to determine the degree of substitution (DS) and reaction efficiency (RE) of the derived OS starches (Bai & Shi, 2011). Unreacted OSA residue was removed from the starch sample (5.0 g, db) by washing twice with 20 mL methanol. The OS starch was then suspended in 20 mL of 0.1 M HCl and stirred at 500 rpm for 30 min. The suspension was filtered, and the starch cake was washed with distilled water until no Cl^- was detected by 0.1 M $AgNO_3$. Distilled water (300 mL) containing 30 μL thermostable α-amylase was used to re-suspend the starch, and the mixture was heated in boiling water bath for 20 min with stirring at 400 rpm. Sodium hydroxide solution (0.100 M) was used to titrate the starch suspension with phenolphthalein as an indicator. Bound OS (%), DS, and RE were calculated using the following equations:

$$\%\mathrm{OS} = \frac{(\mathrm{V_1} - \mathrm{V_2}) \times 0.1 \times 21}{\mathrm{W}} \tag{3.1}$$

$$\mathrm{DS} = \frac{162\times\%\mathrm{OS}}{(210-209)\times\%\mathrm{OS}} \tag{3.2}$$

$$\%\mathrm{RE} = \frac{\%\mathrm{OS\ of\ OS\ starch}}{\%\mathrm{OSA\ added\ to\ the\ starch}} \times 100 \tag{3.3}$$

where %OS was the weight percentage of OS groups in the OS starch, V_1 was the volume (mL) of NaOH that was used to titrate OS starch, V_2 was the volume (mL) of NaOH that was used to titrate the corresponding control starch, and W was the dry weight (g) of the OS starch.

3.2.4 Thermal properties of starch

Thermal properties of the OSA modified starches and the corresponding controls were determined using differential scanning calorimetry (DSC 8000, Perkin Elmer, Woodbridge, ON, Canada) according to the method of Ai et al. (2013) with slight modifications. OS starch (~10 mg) was thoroughly wet with three volumes of distilled water in a stainless-steel pan. After being hermetically sealed, the sample was equilibrated at room temperature for at least 2 h. The starch sample was heated from 10 to 120°C at a rate of 10°C/min, with an empty stainless-steel pan being used as the reference. After the gelatinization, the starch was stored at 4°C for 7 days before the second scan using the same procedure above to measure the melting of retrograded starch. The parameters of thermal transitions (T_o: onset temperature; T_p: peak temperature; T_c: conclusion temperature; and ΔH: enthalpy change) were determined using Pyris software (Perkin-Elmer, Woodbridge, ON, Canada). The percentage of starch retrogradation was calculated using the following equation:

$$\%\mathrm{retrogtadation} = \frac{\Delta\mathrm{H\ of\ melting\ of\ retrograded\ starch}}{\Delta\mathrm{H\ of\ starch\ gelatiniztion}} \times 100 \tag{3.4}$$

3.2.5 Pasting properties of starch

Pasting properties of the OSA modified starches and the corresponding controls were analyzed using a Rapid Visco Analyzer (RVA Super 3, Perten Instruments, Winnipeg, MB, Canada). The starch suspension (8% dry basis, db; 28.0 g total weight) was loaded onto the RVA instrument and run with the Standard Method 2 programmed in the Thermocline Software. Briefly, the suspension was firstly equilibrated at 50°C for 1 min, heated to 95°C at a rate of 6°C/min, kept at 95°C for 5 min, cooled to 50°C at a rate of 6°C/min, and finally kept at 50°C for 2 min. The paddle was rotating at a speed of 960 rpm for the initial 10 s and 160 rpm for the rest period in the analysis (Ai, Medic, Jiang, Wang, & Jane, 2011).

3.2.6 Preparation of OS starch dispersion

OS starch sample was suspended in deionized water (2 wt%, db) with 250 rpm magnetic stirring. The slurry was heated in a boiling water bath (100 °C) under stirring (250 rpm) for 20 min to fully disperse the OS starch, with vigorous manual shaking every 5 min. The obtained starch dispersion was cooled down to room temperature (25 °C) before subsequent experiments. Sodium hydroxide (0.5 M) was used to adjust the pH of the starch dispersion to 7.0. Gum Arabic (GA) dispersion (2 wt%, db) was made by stirring at 250 rpm for 20 min at room temperature, followed by adjusting the pH to 7.0 with 0.5 M NaOH. Gum Arabic was included in the following experiments as the control for comparison.

3.2.7 Interfacial tension between canola oil and OS starch dispersion

The dynamic interfacial tension (IFT) between canola oil and OS starch or GA dispersion was measured by a Force Tensiometer-K20 (Krüss, Germany) using the Wilhelmy plate method (Wang, Ghosh, & Nickerson, 2019). Briefly, OS starch or GA dispersion (23 mL) was added into a glass wide-mouth cup, and the plate was lowered to the location of air-dispersion interface, followed by the careful addition of canola oil (45 mL) on the top of the aqueous dispersion. The force on the plate was recorded by the instrument. IFT was calculated using the Wilhelmy equation:

$$\gamma = \frac{F}{L \times \cos(\theta)} \tag{3.5}$$

where L (40.20 mm) is the length of the plate perimeter, and θ is the contact angle (normally complete wetting, θ = 0). The IFT between the oil and each dispersion was recorded every 5 min for a total period of 2 h.

3.2.8 Emulsion formation using OS starch as the emulsifier

The OS starch or GA dispersion (190 g) containing 4.0 g (db) of the emulsifier was prepared as the aqueous phase prior to the emulsion formation (see Section 3.2.6). Sodium azide was dissolved in the aqueous phase (0.02 wt% of the entire emulsion system) to prevent the growth of microbes. Canola oil (10 g) was added to the aqueous phase and mixed by magnetic stirring (600 rpm) for 1 h. The mixture was homogenized by a rotor mixer (Polytron, Brinkmann instruments, ON, Canada) at 6,000 rpm for 4 min to generate a coarse emulsion, which was then passed through a high-pressure homogenizer (Emulsiflex-C3, Avestin Inc., Ottawa, ON, Canada) at 20,000 psi for 5 cycles to produce a fine oil-in-water (O/W) emulsion. The final emulsion system contained 5 wt%

canola oil and 2 wt% OS starch or GA. The prepared emulsion sample (15 mL) was stored at 4 °C for 28 days to determine the storage stability.

3.2.9 Droplet-size distribution of emulsion

Droplet-size distributions of the fresh and 28-d emulsions were determined by Mastersizer 2000 laser light scattering instrument (Malvern Instruments, Montreal, QC, Canada). The emulsion sample was added to the instrument drop by drop using a disposable pipette until the obscuration reached around 14%. Droplet-size distribution was calculated by Mastersizer 2000 v 5.54 software (Malvern Instruments, Montreal, QC, Canada) based on Mie theory. The relative refractive indices of the canola oil and the continuous aqueous phase were 1.47 and 1.33, respectively. The droplet-size distribution and volume weighted mean droplet diameter ($d_{4,3}$) were reported. The $d_{4,3}$ was calculated using the following equation:

$$d_{4,3} = \frac{\Sigma_{i=1} n_i \times {d_i}^4}{\Sigma_{i=1} n_i \times {d_i}^3} \qquad (3.6)$$

where n_i was the number of droplets of diameter (d_i) (McClements, 2015).

3.2.10 Zeta potential of emulsion

Zeta potential (ξ, mV) of the fresh and 28-d emulsions were measured by Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA, USA). Prior to the test, five drops of the emulsion were diluted in 50 mL Milli-Q deionized water, and the dilution was then injected into a testing cell to determine the electrophoretic mobility (U_E). Henry's equation was used to estimate the zeta potential (ξ):

$$U_E = \frac{2\varepsilon \times \xi \times f(\kappa\alpha)}{3\eta} \qquad (3.7)$$

where, ε was the permittivity, f (κα) was a function related to the Debye length (κ) and the ratio of particle radius (α), and η was the viscosity of the solution. The Smoluchowski approximation f (κα) was set to 1.5 for this study.

3.2.11 Accelerated stability of emulsion

Accelerated stability of the prepared emulsions was determined by using a photocentrifuge dispersion analyzer (LUMiSizer, LUM Americas, Boulder, CO, U.S.A.). The fresh emulsion (400 μL) was injected into a cuvette and centrifuged at 2000 *g* for 16 h. Transmission profile of 865 nm laser through the emulsion sample was recorded every 60 s during the centrifugation. During the centrifugation in the photocentrifuge, the oil droplets moved toward the top of the cuvette and formed a cream layer, the rate of droplet moving was measured as creaming velocity. The transmitted light intensity through the emulsion was changed with the moving of oil droplets under the centrifugal force. The instability index and creaming velocity of the sample were calculated by the installed SEPView software v 4.1 (LUM GmbH, Berlin, Germany) from the final transmission profile (Primozic, Duchek, Nickerson, & Ghosh, 2018).

3.2.12 Effects of pH, sodium chloride and heating on emulsion stability

Emulsion stability against various pH values, NaCl concentrations, and heating treatment was investigated in this thesis.

a. Effect of pH

After the emulsions were prepared as described in Section 3.2.8, different HCl solutions (3.0, 2.0, 1.0, 0.5 and 0.1 M) were used to adjust the pH of the emulsion samples to 2, 3, 4, 5 and 6, respectively. The emulsions at the selected pH values (8 mL each) were transferred into 10-mL glass test tubes and stored at 4 °C overnight for further characterization.

b. Effect of NaCl

Certain amounts of solid NaCl were added to 8 mL emulsion samples at pH 7 to achieve a final salt concentration of 0, 0.1, 0.5 and 1.0 M in 10-mL glass tubes. The tubes were shaken reversely to fully dissolve the added NaCl. The samples were kept at 4 °C overnight for further characterization.

c. Effect of heating

Freshly made emulsions (4 mL each) at pH 7 were transferred into 10-mL glass test tubes. The test tubes were incubated at 90°C in a water bath for 30 min and then kept at 4 °C overnight for further characterization.

The emulsions exposed to the above conditions were characterized by visual observation, determination of droplet-size distribution and zeta potential.

3.2.13 Confocal laser scanning microscopy of emulsion

Confocal laser scanning microscopy (CLSM) was conducted on all the fresh and 28-d emulsions made with OS starches or GA as well as those subjected to the conditions of pH 2, 0.1 M NaCl or heating at 90°C. Nile red (0.01 wt%; excitation by 543 nm laser) was added to the canola oil prior to the mixing homogenization step as described in Section 3.2.8, and Nile blue A (excitation by 633 nm laser) was used to stain the starch molecules. In brief, Nile blue A solution (0.1 wt%) was prepared by dissolving the dye in deionized water under magnetic stir (~200 rpm) overnight, and one drop of this Nile blue A solution was homogeneously mixed with nine drops of the emulsion containing Nile red. One drop of the prepared sample was transferred to a glass slide using a glass rod. After being covered with a coverslip, the slide was loaded onto a Nikon C2 microscope (Nikon Inc., Mississauga, ON, Canada). Nile red and Nile blue A was excited by 543 nm laser and 633 nm laser respectively, and the emission was collected in 573-613 nm range for Nile red and range 650-1000 nm for Nile blue A. The images were taken with a 60 × Plan Apo VC (numerical aperture 1.4) oil immersion objective lens with 5× magnification. For each sample, representative images were taken from three to five different locations on the slide.

3.2.14 Statistical analysis

The OS starch was prepared in three batches independently for one starch and one level of OSA modification in Section 3.2.2. Emulsion was prepared from each batch of OS starch once, and emulsion made with GA was prepared in triplicate in Section 3.2.8. Each sample was measured once in all the analyses. The result was reported as average ± standard deviation (calculate from three replicates/batches of the same starch) unless specifically indicated. The collected data were analyzed using one-way ANOVA test at a significance level of 0.05 to determine the statistical differences among the treatments. The statistical analysis was performed using IBM SPSS Statistics Analysis (Version 22.0).

4. RESULTS AND DISCUSSION

4.1 Starch modification and physicochemical properties of modified starch

4.1.1 Amylose content of native starch

Amylose contents of the native starches were in an ascending order of waxy corn (WCS) < normal corn (NCS) < pea (PS) (Table 4.1), which are in good accordance with previous literature (Li et al., 2019).

Table 4.1 Amylose contents of native pea (PS), normal corn (NCS) and waxy corn starches (WCS)[a].

Starch	Amylose content (%)[b]
PS	41.5 ± 0.6 c
NCS	32.9 ± 0.9 b
WCS	1.7 ± 0.0 a

[a]Data are presented as average ± standard deviation (n = 3); the numbers with different letters are significantly different at $p < 0.05$.

[b]Determined using a potentiometric titration method.

4.1.2 Degree of substitution and reaction efficiency of OS starch

The %OS and DS of the OS starches of the same starch type increased with a higher level of OSA used in the modification (Table 4.2). %OS indicated the weight percentage of OS groups that actually attached to the OS starch in the modification. It is important to note that the %OS value of each OS starch was lower than the %OSA being added to the starch even it was name as "OS (% used for modification) abbr. of starch" [*e.g.*, the %OS value of OS (1%) PS was 0.52%]. As the concentration of added OSA was elevated from 1% to 5%, the DS increased from 0.004 to 0.027 for modified PS, from 0.005 to 0.025 for modified NCS, and from 0.004 to 0.028 for modified WCS, respectively. Similar results have been reported for OSA modification of WCS in a different study (Miao et al., 2014). The RE of OS PS and OS WCS increased with a higher level of added OSA, while the RE of three OS NCS showed no apparent difference. The effect of added OSA concentration on the RE of OS starch being observed in this thesis was different from those reported in previous studies that conduced on OSA modification of NCS (Ai et al., 2013) and rice starch (Bai & Shi, 2011). In addition, at the three levels of OSA used for the modification, the effects of starch types on the %OS, DS and RE did not show clear trends in this study, inconsistent with previous research (Abiddin et al., 2018; Lopez-Silva, Bello-Perez, Agama-Acevedo, & Alvarez-Ramirez, 2019). Those discrepancies could be explained by the starches of various origins used in the different studies.

Table 4.2 OS weight percentages (%OS), degrees of substitution (DS), and reaction efficiencies (RE) of pea (PS), normal corn (NCS), waxy corn starches (WCS) modified with 1%, 3% or 5% (w/w, db) octenyl succinic anhydride (OSA)[a].

Sample	%OS	DS[b]	RE (%)
OS (1%) PS	0.52 ± 0.06 a	0.0040 ± 0.0004 a	51.8 ± 5.6 a
OS (3%) PS	1.96 ± 0.09 b	0.0154 ± 0.0017 b	65.2 ± 3.1 bc
OS (5%) PS	3.41 ± 0.12 d	0.0272 ± 0.0010 d	68.1 ± 2.5 bc
OS (1%) NCS	0.61 ± 0.01 a	0.0047 ± 0.0001 a	60.8 ± 1.0 b
OS (3%) NCS	1.90 ± 0.06 b	0.0149 ± 0.0005 b	63.3 ± 2.1 b
OS (5%) NCS	3.10 ± 0.04 c	0.0247 ± 0.0003 c	62.1 ± 0.7 bc
OS (1%) WCS	0.45 ± 0.03 a	0.0035 ± 0.0003 a	45.3 ± 3.4 a
OS (3%) WCS	2.03 ± 0.06 b	0.0160 ± 0.0005 b	67.8 ± 2.0 bc
OS (5%) WCS	3.52 ± 0.04 d	0.0281 ± 0.0003 d	70.3 ± 0.8 c

[a] Data are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Determined using titration method of Bai and Shi (2011).

4.1.3 Gelatinization and retrogradation properties of OS starch

The thermal properties of the control and OS starches are presented in Table 4.3. The substitution with 1% OSA did not appear to significantly alter the gelatinization temperatures and ΔH of PS, NCS and WCS. Similar behaviors were observed in the gelatinization of OS WCS and OS potato starch at 1% OSA modification (Bhosale & Singhal, 2007; Won et al., 2017). The lack of significant change in the gelatinization behavior of OS (1%) starch was due to the low level of modification. As the level of added OSA increased to 3% and 5%, the gelatinization temperatures and ΔH of all the three starches became lower than their corresponding controls. The data indicated that the OSA modification destabilized the double helices between starch chains in the granules (Ai et al., 2013; Han & BeMiller, 2007).

After gelatinized starch samples were stored at 4°C for 7 days, T_o and T_p of the melting of retrograded OS PS and OS NCS were generally higher than those of their respective controls (Table 4.3), whereas the T_c of the melting of retrograded OS PS and OS NCS were comparable. By contrast, the OSA modification reduced the T_o, T_p and T_c of the melting of retrograded WCS. The reason for the inconsistent trends in the melting temperatures of the retrograded OS starches could be the different starch types. Previous paper reported that retrograded OS tapioca starch (Thirathumthavorn & Charoenrein, 2006) and retrograded OS high-amylose corn starch (Abiddin et al., 2018) displayed higher T_o, T_p and T_c of melting than those of their corresponding controls. However, retrograded OS rice starch (Thirathumthavorn & Charoenrein, 2006) and retrograded OS sago starch (Abiddin et al., 2018) showed lower T_o, T_p and T_c of melting compared to those of their respective controls. Overall, the OSA modification lowered the percentages of retrogradation of all the three starches, with a more obvious decreasing effect being observed at a higher level of OSA modification. The data suggested that the re-association between starch chains during retrogradation was interfered by the presence of the esterified OS groups (Thirathumthavorn & Charoenrein, 2006). For the controls and starches modified with the same concentration of OSA, the percentages of retrogradation of the starches were in a descending order of PS > NCS > WCS. The larger percentages of retrogradation of the control and OS PS could be related to the higher amylose content (Table 4.1) and the longer branch chains of amylopectin of native PS (Li et al., 2019).

Table 4.3 Gelatinization and retrogradation properties of control pea (PS), normal corn (NCS), waxy corn starches (WCS) and those modified with 1%, 3% or 5% (w/w, db) octenyl succinic anhydride (OSA)[a,b].

Sample	Gelatinization of starch[c]				Melting of retrograded starch[c]				Retrogradation
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	(%)[d]
PS-C	62.4 ± 0.2 c	67.9 ± 0.1 c	75.5 ± 0.4 b	16.9 ± 0.5 bc	43.0 ± 0.9 b	58.9 ± 0.2 cd	72.0 ± 0.6 e	8.8 ± 0.4 g	52.2 ± 1.1 f
OS (1%) PS	62.8 ± 1.3 c	67.5 ± 0.2 c	75.0 ± 0.2 ab	16.8 ± 0.8 bc	49.6 ± 0.3 cd	60.2 ± 0.0 de	72.3 ± 1.3 e	7.7 ± 0.7 f	45.7 ± 5.1 e
OS (3%) PS	57.8 ± 0.7 b	64.2 ± 0.3 a	74.9 ± 0.1 a	15.4 ± 0.4 b	49.7 ± 0.8 cd	61.5 ± 0.9 e	71.5 ± 0.8 e	5.7 ± 0.2 e	36.7 ± 2.4 d
OS (5%) PS	55.0 ± 0.6 a	65.1 ± 0.0 b	74.9 ± 0.3 ab	11.4 ± 0.3 a	51.0 ± 0.5 d	61.6 ± 1.1 e	70.9 ± 0.9 e	3.9 ± 0.2 d	34.0 ± 1.0 d
NCS-C	67.3 ± 0.3 ef	71.8 ± 0.0 e	78.7 ± 0.1 cd	17.0 ± 0.5 bc	43.6 ± 1.1 b	55.5 ± 0.4 b	64.9 ± 1.0 b	5.9 ± 0.5 e	35.0 ± 3.6 d
OS (1%) NCS	67.8 ± 0.2 ef	72.2 ± 0.2 e	79.0 ± 0.3 d	16.8 ± 0.4 bc	46.8 ± 1.4 c	56.6 ± 0.3 b	64.9 ± 0.7 b	4.1 ± 0.1 d	24.7 ± 1.0 c
OS (3%) NCS	65.0 ± 0.8 d	71.6 ± 0.5 e	78.6 ± 0.1 cd	15.2 ± 1.4 b	49.3 ± 1.5 cd	57.5 ± 1.0 bc	65.2 ± 1.1 b	2.4 ± 0.4 c	15.4 ± 1.1 b
OS (5%) NCS	61.8 ± 1.5 c	70.9 ± 0.2 d	78.1 ± 0.3 c	13.1 ± 1.2 a	51.6 ± 0.3 d	57.5 ± 0.9 bc	63.6 ± 1.3 b	1.3 ± 0.2 b	9.9 ± 0.3 b
WCS-C	68.4 ± 0.4 ef	76.1 ± 0.2 g	83.2 ± 0.1 f	17.4 ± 0.1 c	47.3 ± 1.8 c	59.1 ± 0.5 cd	66.3 ± 0.6 b	4.3 ± 0.4 d	24.6 ± 2.1 c
OS (1%) WCS	69.1 ± 0.6 f	76.5 ± 0.1 g	83.5 ± 0.1 f	17.0 ± 0.6 bc	50.9 ± 0.0 d	58.9 ± 0.8 cd	65.2 ± 1.6 b	2.6 ± 0.1 c	15.1 ± 0.8 b
OS (3%) WCS	66.3 ± 0.7 de	74.3 ± 0.2 f	81.3 ± 0.2 e	16.7 ± 0.2 bc	41.0 ± 1.3 ab	44.9 ± 0.6 a	49.1 ± 0.8 a	0.2 ± 0.1 a	1.4 ± 0.4 a
OS (5%) WCS	62.3 ± 0.1 c	71.7 ± 0.1 e	79.1 ± 0.1 d	16.1 ± 0.4 bc	39.2 ± 0.3 a	45.1 ± 0.6 a	49.1 ± 0.8 a	0.2 ± 0.1 a	1.4 ± 0.4 a

[a] Data are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Determined using a differential scanning calorimeter.

[c] T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, and ΔH = enthalpy change.

[d] %retrogradation = 100 × (ΔH of dissociation of retrograded starch) / (ΔH of starch gelatinization)

4.1.4 Pasting properties of OS starch

In general, pasting temperatures of all the three starches were reduced and their peak viscosities were increased by the OSA modification, with more noticeable influence being detected for a higher level of OSA usage (Fig. 4.1 and Table 4.4). The notable effects of OSA modification on the pasting properties of the starches were attributed to that the incorporation of OS groups partly destroyed the starch granular structure, which could thus lead to easier water penetration and greater granular swelling at a lower temperature (Sharma, Singh, Yadav, Arora, & Vishwakarma, 2016). Moreover, the negatively charged OS groups on the modified starches could cause repulsion between starch chains to further change the pasting properties of PS, NCS and WCS as described above (Bao et al., 2003; Bhosale & Singhal, 2007). Generally, all the OS starches exhibited higher final viscosities than their respective controls, which could be explained by that the repelling between OS groups enhanced the hydrodynamic volume of dispersed starch molecules during cooling (Zhang, Zhao, & Xiong, 2013). For the controls and starches modified with the same level of OSA, the pasting viscosities of the starches were in an ascending order of PS < NCS < WCS, which corresponds well to the difference in the amylose contents of the three starches (Table 4.1) as amylose tended to restrict the swelling of starch granules for viscosity development during pasting (Li et al., 2019). Overall, the observed impact of OSA modification on the pasting properties of the three starches is in good agreement with previous findings on OS starches of other botanical origins, such as rice (Song et al., 2006) and pearl millet (Sharma et al., 2016).

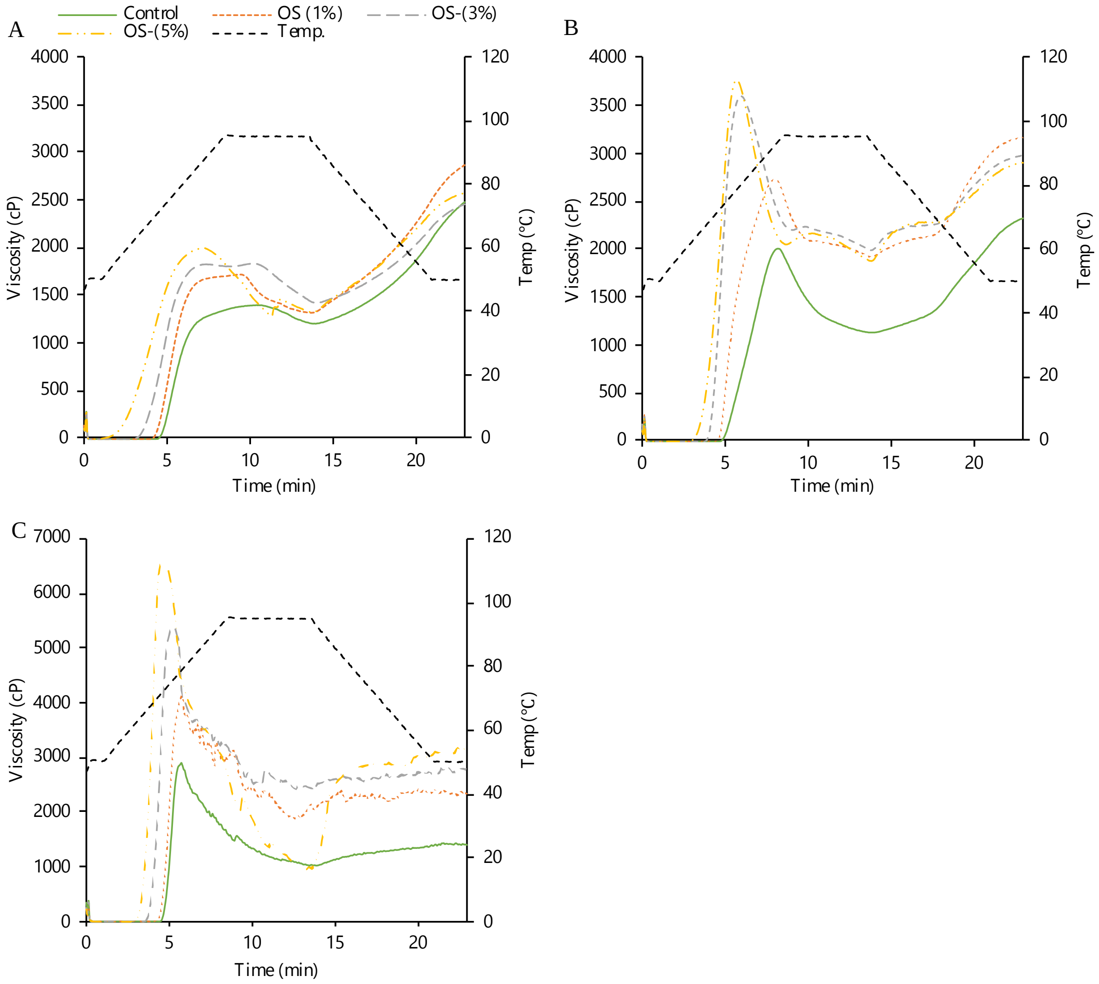


Fig. 4.1. Pasting curves of control starches and those modified with 1%, 3% or 5% (w/w, db) octenyl succinic anhydride (OSA). A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS). The scale of y axis in C was different from those in A and B. Starch suspensions (28 g total weight containing 8% starch, db) were analyzed using RVA Super 3 in the experiment.

Table 4.4 Pasting properties of control pea (PS), normal corn (NCS), waxy corn starches (WCS) and those modified with 1%, 3% or 5% (w/w, db) octenyl succinic anhydride (OSA)[a,b].

Sample	Pasting temperature (ºC)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback (cP)
PS-C	72.1 ± 0.3 hi	1396 ± 7 a	1200 ± 41 ab	195 ± 40 a	2466 ± 32 b	1266 ± 17 bc
OS (1%) PS	69.6 ± 0.0 f	1719 ± 27 b	1318 ± 16 ab	401 ± 40 ab	2859 ± 80 d	1541 ± 68 c
OS (3%) PS	64.9 ± 0.5 cd	1853 ± 120 bc	1422 ± 62 bc	431 ± 153 abc	2463 ± 107 b	1041 ± 115 b
OS (5%) PS	57.5 ± 1.1 a	1992 ± 170 bc	1318 ± 81 ab	674 ± 89 bc	2565 ± 140 bc	1247 ± 59 bc
NCS-C	73.4 ± 0.3 i	1999 ± 37 c	1129 ± 9 ab	870 ± 28 c	2315 ± 30 b	1186 ± 22 bc
OS (1%) NCS	71.9 ± 0.0 h	2738 ± 42 d	1925 ± 132 d	813 ± 91 bc	3177 ± 65 f	1252 ± 72 bc
OS (3%) NCS	67.7 ± 0.4 e	3591 ± 114 e	1984 ± 36 d	1606 ± 149 d	2969 ± 61 def	985 ± 27 b
OS (5%) NCS	63.6 ± 0.8 bc	3767 ± 70 e	1878 ± 220 d	1889 ± 142 d	2903 ± 140 de	1025 ± 121 b
WCS-C	71.4 ± 0.2 gh	2933 ± 89 d	1008 ± 30 ab	1925 ± 119 d	1394 ± 45 a	386 ± 74 a
OS (1%) WCS	70.1 ± 0.3 fg	4771 ± 106 f	1801 ± 384 cd	2370 ± 366 e	2341 ± 89 b	539 ± 425 a
OS (3%) WCS	65.9 ± 0.3 d	5377 ± 139 g	2495 ± 44 e	2972 ± 122 f	2774 ± 45 cd	369 ± 69 a
OS (5%) WCS	62.3 ± 0.4 b	6648 ± 48 h	945 ± 91 a	5703 ± 138 g	3128 ± 101 ef	2182 ± 172 d

[a] Data are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Determined using RVA Super 3.

4.2 Emulsifying property of OS starch and stability of OS-starch emulsion

4.2.1 Interfacial properties of OS-starch dispersion

As the standard emulsifier used in the food industry, GA reduced the oil-water (O/W) interfacial tension (IFT) from 21.1 to 4.2 mN/m during the 2-h test. The IFT dropped remarkably in the initial 10 min and soon reached a plateau (Fig. 4.2). All the tested OS-starch dispersions were capable of reducing the IFT between oil and water. But the IFT was decreased at a considerably slower rate than GA and did not reach a plateau within the 2 h period (except for OS (3%) WCS and OS (5%) WCS), which could be related to the slow migration of starch molecules from the aqueous dispersion to the interface as a result of high viscosity (final viscosity in Table 4.4). All the OS-starch dispersions exhibited IFT smaller than those of their respective controls (data not shown), indicating that the OS starches possessed stronger surface activity because of the improved amphiphilic property after the introduction of hydrophobic OS groups (Królikowska, Pietrzyk, Fortuna, Pająk, & Witczak, 2019).

Among the OS starches of the three origins, they lowered the IFT at different rates. Overall, OS WCS decreased the IFT at the highest rate, followed by OS NCS and OS PS (Fig. 4.2). At the end of the 2 h period, the IFT values were reduced by OS WCS and OS NCS to a level similar to that of GA dispersion, whereas those of OS PS were mostly higher than those of the other OS-starch dispersions. The results suggested that the surface activities of the OS starches were in a descending order of OS WCS ≈ OS NCS > OS PS. For the OS starches from the same original type, the difference among the IFT values at 2 h was insignificant despite lower IFT values being observed in OS (3%) and OS (5%) starch dispersions. The trend is inconsistent with the findings reported for OS WCS (Zhao et al., 2018) and OS NCS (Królikowska et al., 2019), in which greater DS tends to result in better surface activity of OS starch. The discrepancy could be caused by the different concentrations of OS starch being used in the emulsions.

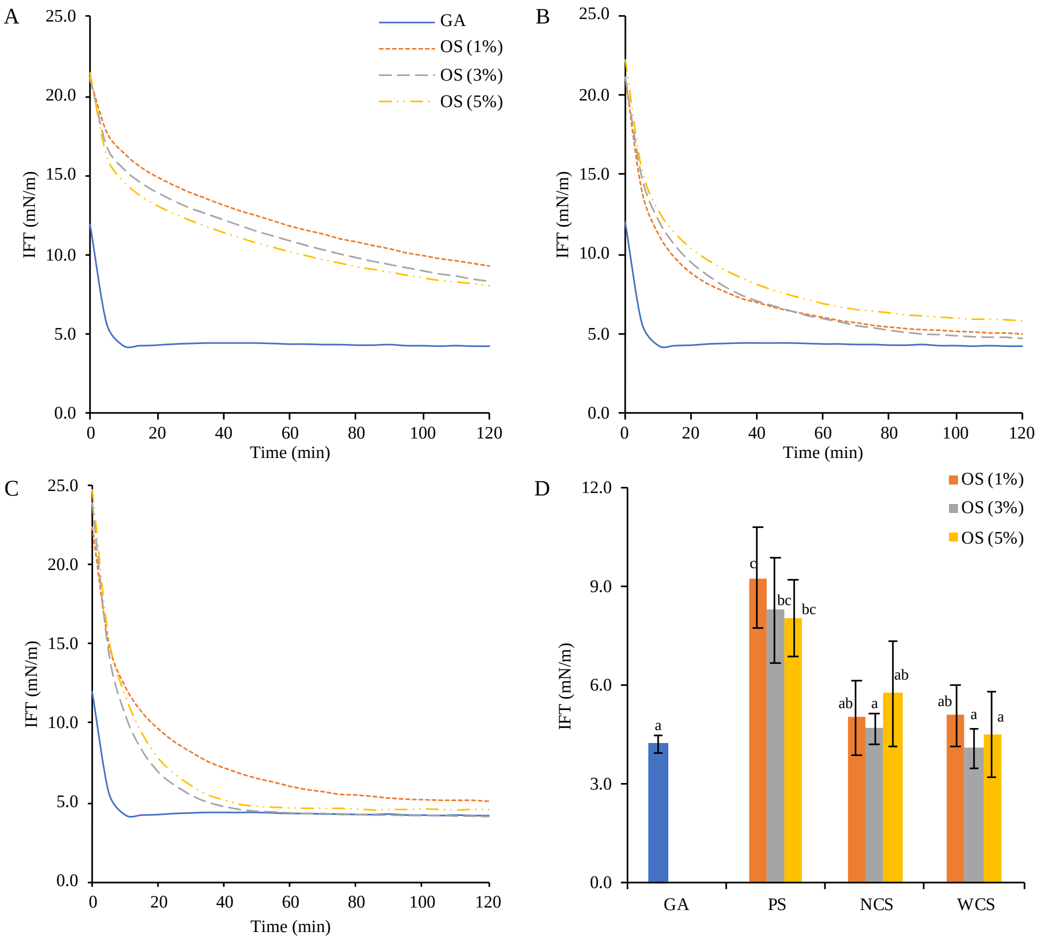


Fig. 4.2. Interfacial tension (IFT) between canola oil and water with gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or with OS starch (2 wt%, db, in aqueous dispersion) being used as the emulsifier. A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS). D: IFT of the examined dispersions at the end of 2 h testing period; among all the measured dispersions, data with the same letter are not significantly different at $p < 0.05$.

4.2.2 Droplet-size distribution, zeta potential and microstructure of fresh OS starch stabilized emulsion

The emulsions freshly prepared with the OS starches possessed droplet diameters ($d_{4,3}$) ranging from 0.221 to 0.321 µm, which were smaller than that of emulsion made with GA (0.571 µm; Fig. 4.3). The droplet-size-distribution (DSD) curve of the emulsion prepared with GA showed a major peak (from 0.1 to 2.5 µm) to the right of the major peak (from 0.05 to 1 µm) in the DSD curves of OS-starch emulsions (Fig. 4.4). The $d_{4,3}$ results are in accordance with those reported by Charoen et al. (2011), in which droplet diameters of emulsions developed with OS starches were considerably smaller than that of GA emulsion.

The fresh emulsion formed with OS (1%) PS displayed a larger $d_{4,3}$ (0.321 µm) compared to the emulsions stabilized by OS (3%) PS (0.221 µm) and OS (5%) PS (0.240 µm), despite the difference being insignificant (Fig. 4.3. A-1). The DSD curves of OS-PS emulsions exhibited a bimodal distribution: a major peak between 0.04 and 1 µm and a minor one between 1 and 10 µm (Fig. 4.4. A-1). The minor peak of 1-10 µm gradually reduced as the level of OSA modification increased from 1% to 5%. The DSD curves of OS-PS emulsions explained that the larger $d_{4,3}$ of OS (1%)-PS emulsion was attributed to the higher relative volume of large droplets of 1-10 µm. In contrast, emulsions prepared with OS NCS and OS WCS displayed comparable droplet-size distributions and $d_{4,3}$ (0.269-0.300 µm and 0.225-0.264 µm, respectively). The OS NCS and OS WCS emulsions exhibited a major peak (of 0.04-1.6 µm for OS NCS and of 0.03-1.2 µm for OS WCS, respectively) in their DSD curves, except that a very small peak of 1-10 µm was found in OS (1%)-NCS and OS (1%)-WCS emulsions (Fig. 4.4. B-1 and C-1).

For the three starches modified with the same concentration of OSA, the $d_{4,3}$ of the resultant fresh emulsions generally showed an ascending order of OS WCS $\approx$ OS PS < OS NCS. It is interesting to note that overall the three levels of OSA modification did not significantly affect the $d_{4,3}$ of emulsions prepared with the OS starches of the same botanical origin in this study, which was in agreement with the IFT results at 2 h (Fig. 4.2. D). In contrast, the research performed by Lin, Liang, Zhong, Ye, and Singh (2018a) showed an increasing droplet size of emulsion as the DS of OS WCS starch increased. The authors related the phenomenon to: (1) the enhanced steric hindrance of interfacial layer by the greater concentration of OS groups in the modified starch; (2) more viscous OS-WCS dispersion with higher DS reduced the efficiency of microfluidizer during homogenization of emulsions (Wang, Su, & Wang, 2010). The different observation was possibly due to the relative high concentrations of OSA used (5%, 7% and 9%) in the study of Lin et al. (2018a).

The different size distributions of the droplets in the GA and OS-starch emulsions are consistent with the observation in CLSM images (Fig. 4.5). A few large droplets observed in the CLSM images (*e.g.*, GA-0 d) were in accordance with the coverage of DSD curves of the emulsions (Fig. 4.4 Day 0). The majority of the droplets in the images of OS-starch emulsions were smaller than those in GA emulsion. In the CLSM images, oil droplets were found to be covered with emulsifier molecules (green layer), and the overlapped area between two droplets displayed yellow color under laser scanning (indicated by blue arrows). The differences among $d_{4,3}$ of droplets in the images of OS-starch emulsions were hard to differentiate visually, which may due to the $d_{4,3}$ of those emulsions were beyond the detecting limitation of the instrument. However, the droplets in images of OS-WCS emulsions tended to be smaller than those in OS-NCS and OS-PS emulsions using OS starch with the same level of OSA modification.

Freshly prepared GA emulsion displayed a negative surface charge of -67.7 mV (Fig. 4.3) due to the existence of anionic polysaccharides in GA (Renard et al., 2006). Zeta potentials of all the OS-starch emulsions ranged from -38.3 to -55.6 mV (Fig. 4.3. A-2, B-2, C-2), and the negative surface charge was because of the presence of OS groups. The absolute surface charge values of the emulsions increased when the used OSA concentration was elevated from 1% to 5% for the same starch type due to more OS groups being attached to the starch molecules (Table 4.2), although the difference among the three modified starches within the same group was insignificant. The results are in good agreement with the data reported by Miao et al. (2014). The greater absolute surface value suggested greater electrostatic repulsion among oil droplets and longer distance between two emulsion droplets, which led to less tendency to aggregation (Thaiphanit, Schleining, & Anprung, 2016). Therefore, the emulsions produced by OS starch with higher DS tended to exhibit better physical stability due to stronger repulsion forces. The effect of zeta potential on emulsion stability will be discussed thoroughly in Section 4.2.5 to 4.2.7.

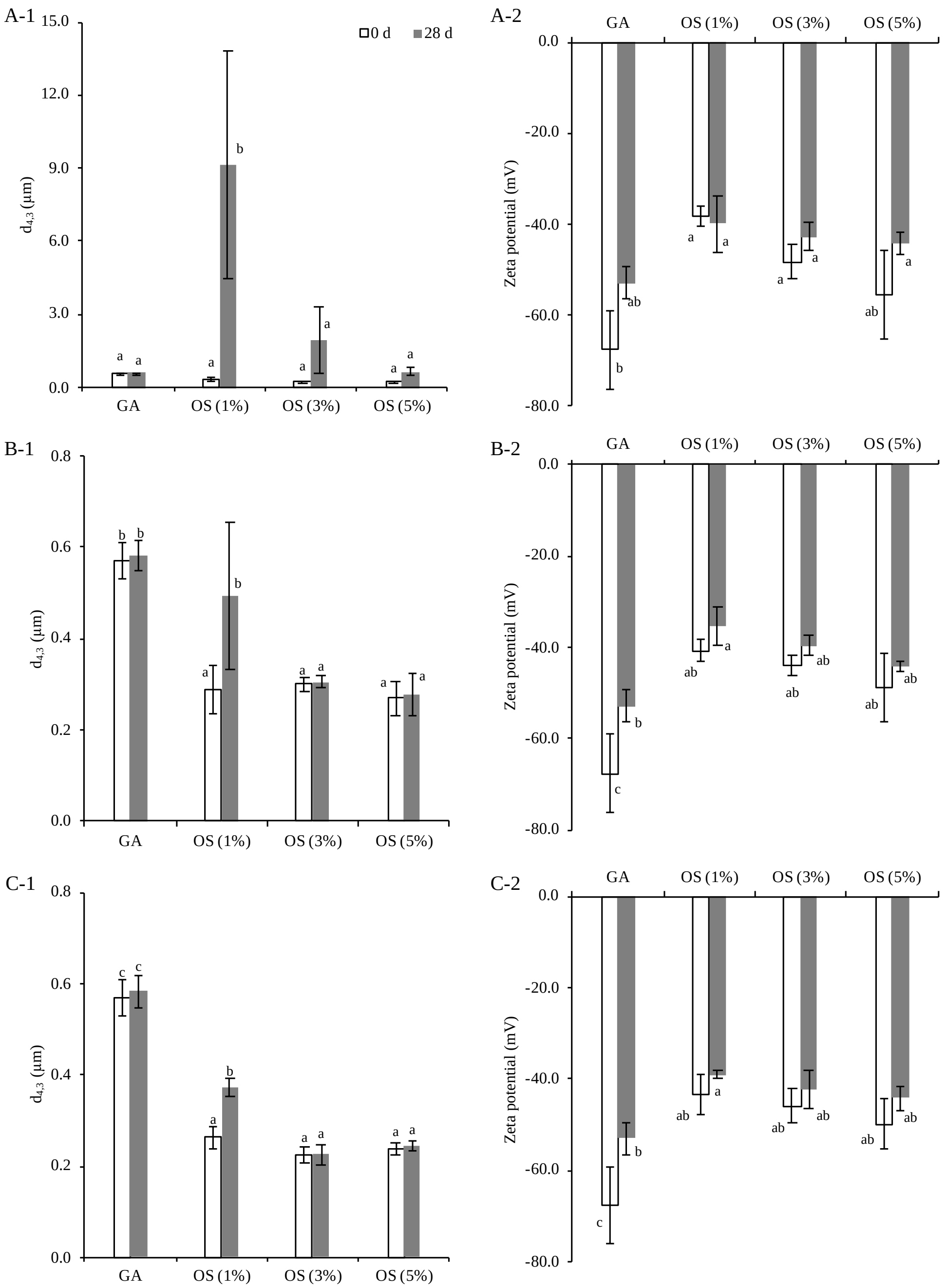


Fig. 4.3. Volume weighted mean droplet diameter ($d_{4,3}$) and zeta potential of fresh (0 day) and 28-d emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion). A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS). The scale of y axis in A-1 was different from those in B-1, C-1. Statistical analysis was conducted among the data obtained from all the eight emulsions (0 and 28 days) presented in the same sub-figure (*e.g.*, A-1, B-1, C-1, A-2, B-2 and C-2). In each sub-figure, data with the same letter are not significantly different at $p < 0.05$.

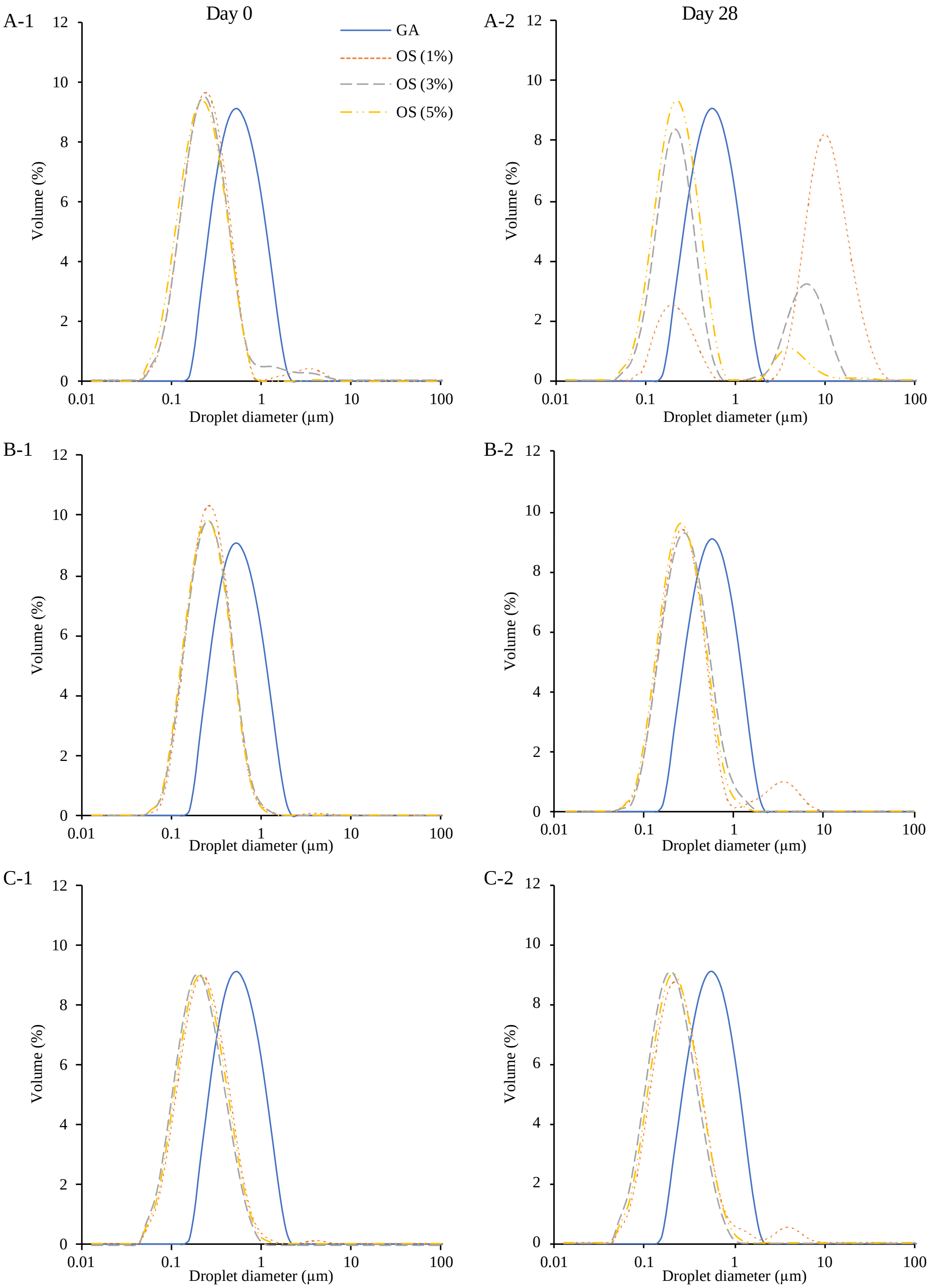
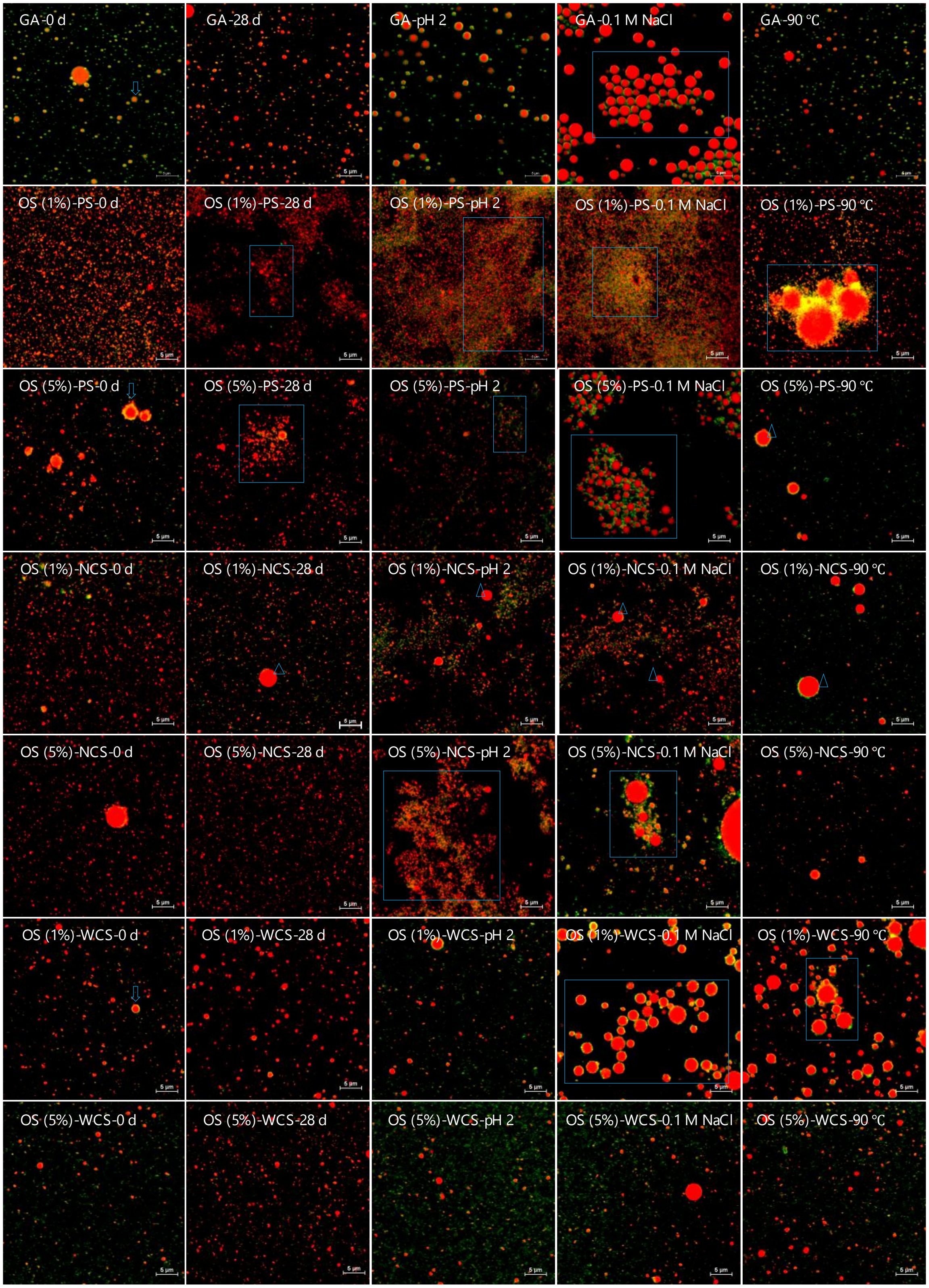


Fig. 4.4. Droplet-size distribution (DSD) curves of emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion) on day 0 and day 28. A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS).


GA-0 d
GA-28 d
GA-pH 2
GA-0.1 M NaCl
GA-90 ℃
OS (1%)-PS-0 d
OS (1%)-PS-28 d
OS (1%)-PS-pH 2
OS (1%)-PS-0.1 M NaCl
OS (1%)-PS-90 ℃
OS (5%)-PS-0 d
OS (5%)-PS-28 d
OS (5%)-PS-pH 2
OS (5%)-PS-0.1 M NaCl
OS (5%)-PS-90 ℃
OS (1%)-NCS-0 d
OS (1%)-NCS-28 d
OS (1%)-NCS-pH 2
OS (1%)-NCS-0.1 M NaCl
OS (1%)-NCS-90 ℃
OS (5%)-NCS-0 d
OS (5%)-NCS-28 d
OS (5%)-NCS-pH 2
OS (5%)-NCS-0.1 M NaCl
OS (5%)-NCS-90 ℃
OS (1%)-WCS-0 d
OS (1%)-WCS-28 d
OS (1%)-WCS-pH 2
OS (1%)-WCS-0.1 M NaCl
OS (1%)-WCS-90 ℃
OS (5%)-WCS-0 d
OS (5%)-WCS-28 d
OS (5%)-WCS-pH 2
OS (5%)-WCS-0.1 M NaCl
OS (5%)-WCS-90 ℃
5 μm

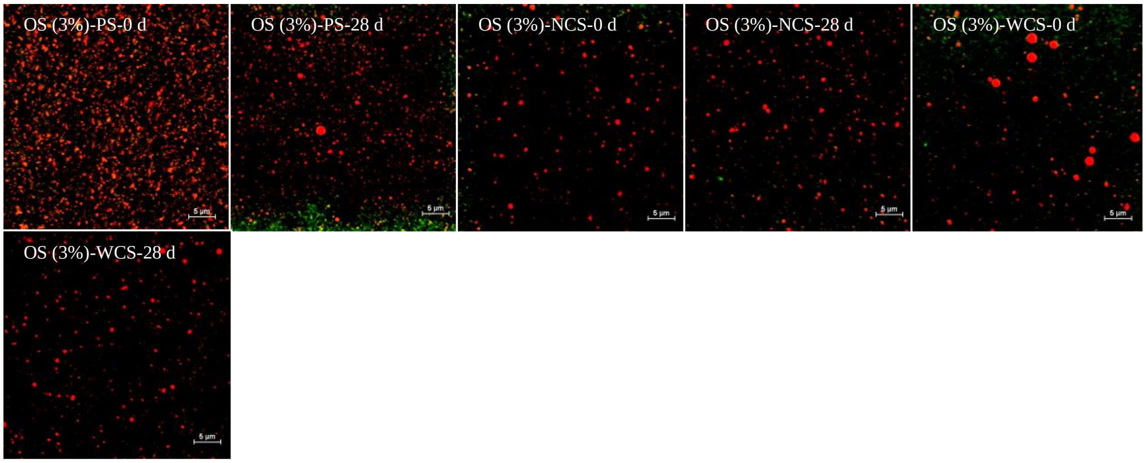


Fig. 4.5. Confocal laser scanning microscopy (CLSM) images of emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion). Canola oil was stained with Nile red, and OS starch was stained with Nile blue A. Scale bar in the image is 5 µm. PS: pea starch; NCS: normal corn starch; and WCS: waxy corn starch. Arrow: starch layer on droplet; triangle: large droplets; rectangle: droplet aggregation.

4.2.3 Accelerated storage stability of OS starch stabilized emulsion

Emulsions produced with OS starches displayed instability indices of 0.83 to 0.92, which was equal or slightly lower than that of GA emulsion (0.92) (Fig. 4.6. A). The results suggested that OS-starch emulsions typically exhibited better stability than GA emulsion under accelerating condition. The statistical analysis revealed that among the three OS starches from the same botanical source, the instability indices of the resulting emulsions were insignificant. The results suggested the similar stability of the emulsions stabilized by the starches modified with 1%-5% OSA. However, emulsions prepared with OS (3%) WCS and OS (5%) WCS showed the lowest instability indices (0.83) among all the samples, suggesting that these two OS starches were able to stabilize the formed emulsions to the greatest extent during accelerated.

The creaming velocity of droplets in GA emulsion was 3.13 μm/s, markedly faster than those of the droplets in emulsions stabilized by OS starches (1.05-1.47 μm/s) (Fig. 4.6. B). The slower movement of droplets in OS-starch emulsions indicated the stronger stability of these emulsions than that of GA emulsion under acceleration. No significant difference was found among the droplet velocity values of the OS-starch emulsions. According to Stokes law (McClements, 2015), emulsion droplet velocity is positively correlated with droplet diameter and negatively correlated with viscosity. Therefore, GA emulsion, having significantly larger droplet size (Fig. 4.3) and lower viscosity (Shah & Singhal, 2018), showed greater creaming velocity.

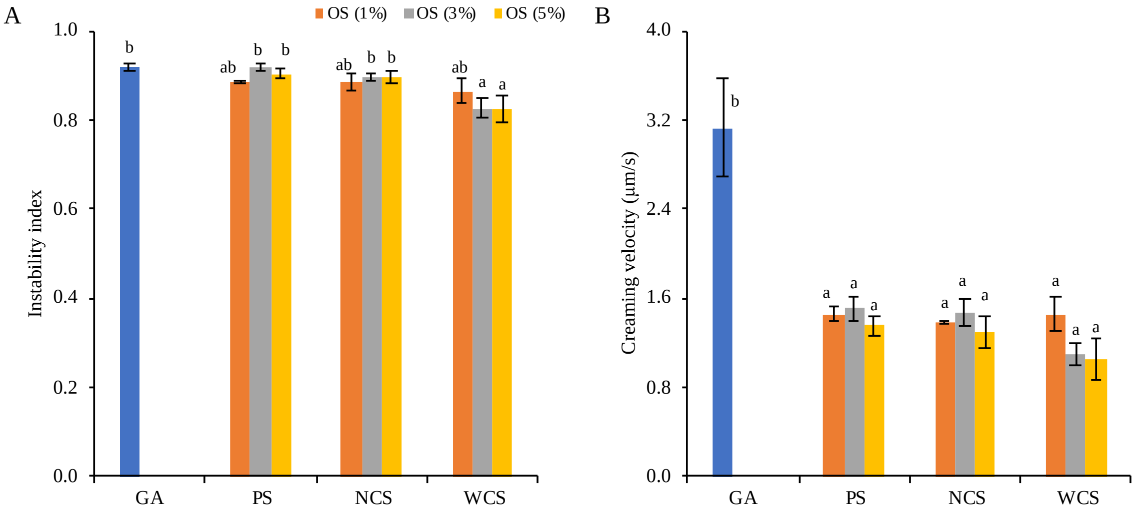


Fig. 4.6. (A) Instability indices and (B) creaming velocity at 2000 *g* of fresh emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion). PS: pea starch; NCS: normal corn starch; and WCS: waxy corn starch. Statistical analysis was conducted among the data obtained from all the ten emulsions in the same sub-figure (*e.g.*, A and B). In each sub-figure, data with the same letter are not significantly different at $p < 0.05$.

4.2.4 Storage stability of OS starch stabilized emulsion

Stability of the developed emulsions after 28-d storage under 4 °C conditions was also assessed, and the data are presented in Fig. 4.3, Fig. 4.4 and Fig. 4.5. The $d_{4,3}$ (0.582 µm) and DSD curve of GA emulsion stored for 28 d showed no apparent change from those of fresh counterpart ($d_{4,3}$ = 0.571 µm) (Fig. 4.3 and 4.4). Droplets with small diameter were found in the captured CLSM images of stored GA emulsions, comparable to those small droplets in the image of fresh GA emulsion (Fig. 4.5).

In comparison with GA emulsion, the emulsions developed with OS PS showed poorer stability upon 28-d storage. The $d_{4,3}$ of OS-PS emulsions on day 28 (0.657-9.147 µm) were larger than that of GA emulsion (0.582 µm; Fig. 4.3. A-1). There was a drastic increment in the $d_{4,3}$ of OS (1%)-PS emulsion after storage (from 0.321 to 9.147 µm). Although the changes in the $d_{4,3}$ of OS (3%)-PS and OS (5%)-PS emulsions before and after storage were insignificant, a general trend of increment was observed. Fig. 4.4. A-2 presented the DSD curves of OS-PS emulsions, the storage noticeably increased the relative volume of the peak corresponding to the droplets of larger sizes, with a smaller extent of increase as the DS increased. The data indicated the poorest stability of OS-PS emulsions of all the samples during storage.

The behaviors of the emulsions prepared with OS NCS and OS WCS upon 28-d storage were similar. The $d_{4,3}$ of the emulsions made with OS (1%) NCS and OS (1%) WCS significantly increased after 28-d storage (Fig. 4.3. B-1, C-1), which was attributed to the increment in the relative volume of large droplets of 1-10 µm as shown in the DSD curves (Fig. 4.4. B-2, C-2). By contrast, no significant change was observed between 0 and 28 d in the $d_{4,3}$ of emulsions formed with NCS and WCS modified with 3% and 5% OSA as their DSD curves largely remained the same before and after storage. Consequently, the $d_{4,3}$ of OS-NCS emulsions (0.277-0.495 µm) and OS-WCS emulsions (0.225-0.371 µm) on 28 d remained smaller than that of GA emulsion (0.582 µm). The stability of the OS (3%)-NCS/WCS and OS (5%)-NCS/WCS emulsions against storage is in good agreement with previous studies, in which no obvious oil separation and $d_{4,3}$ change were observed in the emulsion formed with 3% OSA modified WCS after 20-d storage (Liu et al., 2008) and in emulsion formed with 5% OSA modified WCS after 30-d storage (Zhao et al., 2018).

Overall, OS PS displayed the poorest ability in stabilizing O/W emulsion against storage of all the three OS starch types. For the same origin of starch, starches modified with 3% and 5% OSA exhibited stronger capability of stabilizing O/W emulsion during storage than the counterparts modified with 1% OSA. The findings from the 28-d storage experiment are discrepant from the large instability indices (0.83-0.92) of the OS-starch emulsions measured in the accelerated storage experiment using photocentrifuge (Fig. 4.6. A). The discrepancy could be attributed to the different experimental conditions. The 28-d storage stability of the emulsions was measured under gravity at 4°C, while their accelerated storage stability was determined under centrifugation (2000 *g*) at 25°C. The severe conditions applied in the latter experiment more easily caused disruption of oil droplets and destabilized the emulsions. Additionally, it might be not long enough for 28 d to cause destabilization in all the emulsions.

To further investigate whether the destabilization of emulsions produced with OS PS, OS (1%) NCS and OS (1%) WCS after 28-d storage (Fig. 4.3. A-1, B-1, C-1) was because of droplets aggregation or coalescence, sodium dodecyl sulfate solution (SDS, 1 wt%, pH 7) was used to dilute the samples at a ratio of 1:10 (v/v), and their DSD was measured for the second time. The DSD curves of the tested 28-d emulsions retained the two peaks as displayed in Fig. 4.4. A-2, B-2, C-2, with only a slight decrease in the relative volume of the peak corresponding to the larger droplets (data not shown). The results indicated that coalescence of small droplets into larger ones occurred in the emulsions during storage, possibly with a low extent of aggregation. This statement was supported by the CLSM images of aforementioned 28-d emulsions (Fig. 4.5). Large droplets (indicated by triangle) or droplet aggregation (indicated by rectangle) was found in the images of the aforementioned destabilized emulsions, whereas the droplets in the images of stable emulsions showed similar droplet size before and after storage.

The surface charges of all the 28-d emulsions ranged from -35.4 to -53.0 mV (Fig. 4.3. A-2, B-2, C-2), and the absolute values were smaller than those of their respective fresh counterparts (Fig. 4.3. A-1, B-1, C-1), suggesting changes in conformation of starch molecules on the oil droplet surface. It has been suggested that absolute surface charge above approximate 30 mV could generally prevent droplet flocculation in emulsions stabilized by biopolymers (Mirhosseini, Tan, Hamid, & Yusof, 2008; Najafi, Hosaini, Mohammadi-Sani, & Koocheki, 2016). The absolute surface charges of all the 28-d emulsions were higher than 30 mV, which indicated that electrostatic repulsion among droplets was sufficient to prevent the droplets form aggregation, thus stabilizing the emulsions (Jain, Winuprasith, & Suphantharika, 2019). However, destabilization during storage occurred in several OS-starch emulsions. For OS-PS emulsions, the poorest stability could be partly attributed to the higher amylose content (Table 4.1) and percentage retrogradation of OS PS (Table 4.3). The OS PS molecules existing in the oil-water interface could re-associate and retrograde faster and partially reform the crystalline structure during storage, which destabilized the starch layer surrounding oil droplet. The destabilization of starch layer in OS-PS emulsions during 28-d storage weakened the steric hindrance among oil droplets and thus led to increments in $d_{4,3}$ at Day 28 (Fig. 4.3. A-1). The destabilization observed in OS (1%)-NCS and OS (1%)-WCS emulsions could be due to the smaller DS and greater percentage of retrogradation in comparison with their corresponding counterparts modified with 3% and 5% OSA (Table 4.2 and 4.3).

4.2.5 Effect of pH changing on OS-starch emulsion stability

The $d_{4,3}$ of GA emulsion remained stable when the pH dropped from 7 to 4, and an increase was observed when the pH further dropped to 3 and 2 (Fig. 4.7. A-1). The negative surface charge of GA emulsion gradually changed from -67.7 to -30.6 mV as the pH decreased from 7 to 3 and suddenly changed to -6.7 mV as pH dropped to 2 due to protonation (Fig. 4.7. A-2). As described in Section 4.2.4, an absolute zeta potential larger than 30 mV is generally considered to prevent droplets from aggregation (Mirhosseini et al., 2008; Najafi et al., 2016). Therefore, the reduction in the absolute surface charge value to 30.6 and 6.7 mV at pH 3 and 2 was responsible for the increment in $d_{4,3}$ of GA emulsion. It was reported that GA emulsion was stable over a pH range of 3 to 8 because of the steric repulsion provided by the relatively thick layer of GA coated on the droplet surface (Charoen et al., 2011). The different trend found in the present study could be attributed to that the concentration of GA used (2 wt%) was noticeably lower than that used in the previous study (10 wt%). Compared with the counterpart at pH 7 (Fig. 4.4), the DSD curve of GA emulsion at pH 2 shifted to a large diameter value and possessed a major peak of 1-10 µm (Fig. 4.8). The droplets occurring in the CLSM image of GA emulsion at pH 2 were larger than those of fresh emulsion (Fig. 4.5), corresponding well with the changes describe above.

The $d_{4,3}$ of the three OS-PS emulsions did not significantly change over pH 7-5, but the values increased to different extents as the pH dropped to 4-2 (Fig. 4.7. A-1), with the largest extent of increase being observed with OS (1%)-PS emulsions. The results suggested that OS (1%) PS exhibited the least ability in stabilizing emulsion against pH change among the three OS PS. At the pH of 4-2, OS-PS emulsions exhibited larger $d_{4,3}$ than that of GA emulsion. It is noteworthy that the emulsion prepared with OS (3%) PS generally remained stable over the set pH range of 7-2, despite the slight increase in $d_{4,3}$ at pH 3 and 2.

The trend of change in the $d_{4,3}$ of OS-NCS emulsions under the tested pH was similar to that of OS-PS emulsions. No significant change in the $d_{4,3}$ of OS-NCS emulsions was observed as the pH was decreased from 7 to 4, while the $d_{4,3}$ increased considerably at pH 3 and 2 for OS (3%)-NCS and OS (5%)-NCS emulsions (Fig. 4.7. B-1). It is interesting to note that OS (1%)-NCS emulsion showed good stability over pH 7-2. Generally, the $d_{4,3}$ of OS-NCS emulsions at pH 7-4 appeared to be smaller than that of GA emulsion, however, the $d_{4,3}$ of OS (3%)-NCS and OS (5%)-NCS emulsions became comparable to that of GA emulsion at pH 3 and 2.

The increments in the $d_{4,3}$ of emulsions prepared with OS PS and OS NCS at low pH conditions (4-2) are consistent with the data reported by Zhao et al. (2017) on the emulsions formed with OSA modified kudzu starch. The DSD curves of OS-PS and OS-NCS emulsions at pH 2 showed a bimodal distribution (Fig. 4.8. A and B) instead of one predominant peak at pH 7 (Fig. 4.4. A-1 and B-1), and the presence of the large droplets of 1-100 µm contributed to the increased $d_{4,3}$ of the emulsions (Fig. 4.7. A-1 and B-1). The occurrence of large droplets and droplet aggregation of OS-PS and OS-NCS emulsions at pH 2 were confirmed in the CLSM images, indicating the destabilization of the emulsion systems (Fig. 4.5, the third column).

Interestingly, emulsions developed with OS WCS were highly stable under the tested pH range as their $d_{4,3}$ did not change significantly (Fig. 4.7. C-1). At the same pH, the $d_{4,3}$ of OS-WCS emulsions remained smaller than that of GA emulsion, indicating that OS WCS possessed stronger capability of stabilizing O/W emulsion under pH change compared with GA. The results obtained from this study are consistent with the research conducted by Charoen et al. (2011), in which pH change showed negligible effect on the $d_{4,3}$ of the emulsion stabilized by commercial OS WCS. The good stability of OS-WCS emulsions at pH 2 was confirmed by the lack of apparent shift in their DSD curves (Fig. 4.8. C) and a similar droplet size in CLSM images (Fig. 4.5) when compared with the fresh emulsion at pH 7 (Fig. 4.4. C-1 and Fig. 4.5).

The zeta potentials of the OS-starch emulsions changed in a pattern similar to that of GA emulsion as the pH dropped from 7 to 2 (Fig. 4.7. A-2, B-2, C-2). The absolute surface charges of OS-starch emulsions gradually decreased as pH dropped due to the protonation effect of carboxyl groups in the OS starch, which failed to provide sufficient electrostatic repulsion to keep the emulsion stable at $pH \leq 4$. Consequently, the $d_{4,3}$ of OS-PS and OS-NCS emulsions markedly increased at $pH \leq 4$ (Fig. 4.7. A-1, B-1). It has been demonstrated that the steric repulsion provided by the thick layer of OS starch on droplets is the main mechanism for OS starch to stabilize emulsion (Tesch, Gerhards, & Schubert, 2002). The stability of OS-WCS emulsions against the decrease in pH was attributed to the strong steric repulsion provided by the amylopectin of OS WCS (consisting of 98.3% amylopectin, Table 4.1) (Zhao et al., 2017). The highly branched amylopectin molecules could not easily re-associate with each other, and thus they were able to maintain the strong steric repulsion to stabilize droplets in the emulsions. On the contrary, PS and NCS contained 41.5% and 32.9% amylose (Table 4.1), resulting in less steric repulsion among oil droplets in the resultant emulsions. In addition, higher amylose in PS and NCS could lead to stronger molecular re-association and retrograde at a higher rate (Table 4.3), and these physical changes might cause destabilization of the starch layer on the interface, thus weakening the steric hindrance. As the steric repulsion and surface charge became less strong at low pH (4-2), the oil droplets in OS-PS and OS-NCS emulsions tended to contact with each other and eventually coalesced.

Overall, emulsions developed with the OS starches displayed similar stability against pH change from 7 to 5; however, only those prepared with OS WCS could remain stable as the pH further drop to 4-2. The stability of emulsions formed with OS starches under the tested pH range in this research followed an ascending order of OS PS < OS NCS < OS WCS. The starches modified with 3% and 5% OSA tended to possess stronger ability to stabilize O/W emulsions at pH 7-2 when compared with the corresponding OS (1%) starches.

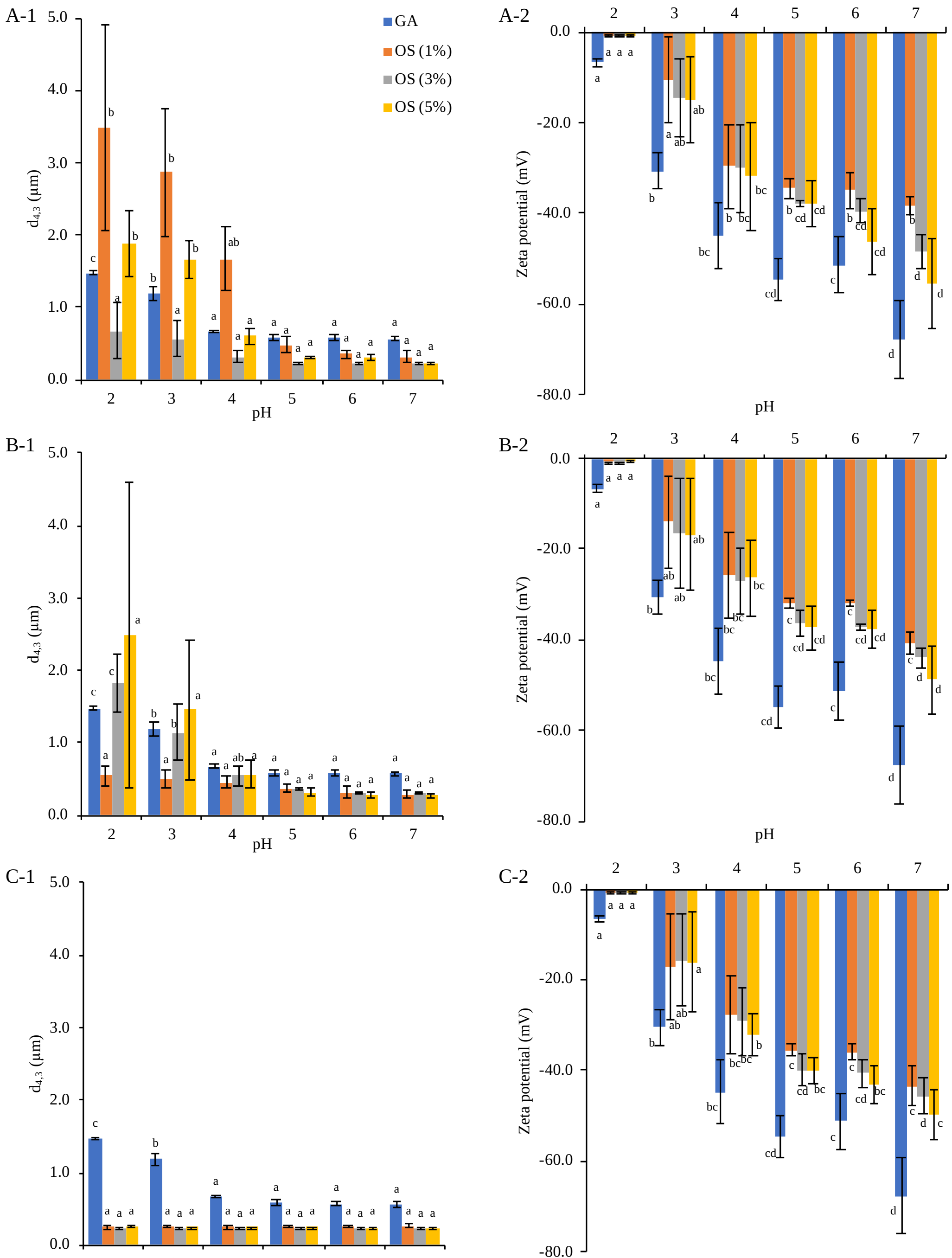


Fig. 4.7. Volume mean droplet diameters ($d_{4,3}$) and zeta potentials of emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion) at pH 2-7. A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS). Statistical analysis was conducted among the data obtained from each emulsion at pH 2-7 in each sub-figure (*e.g.*, A-1, B-1, C-1, A-2, B-2 and C-2). For each emulsion in each sub-figure, data with the same letter are not significantly different at $p < 0.05$.

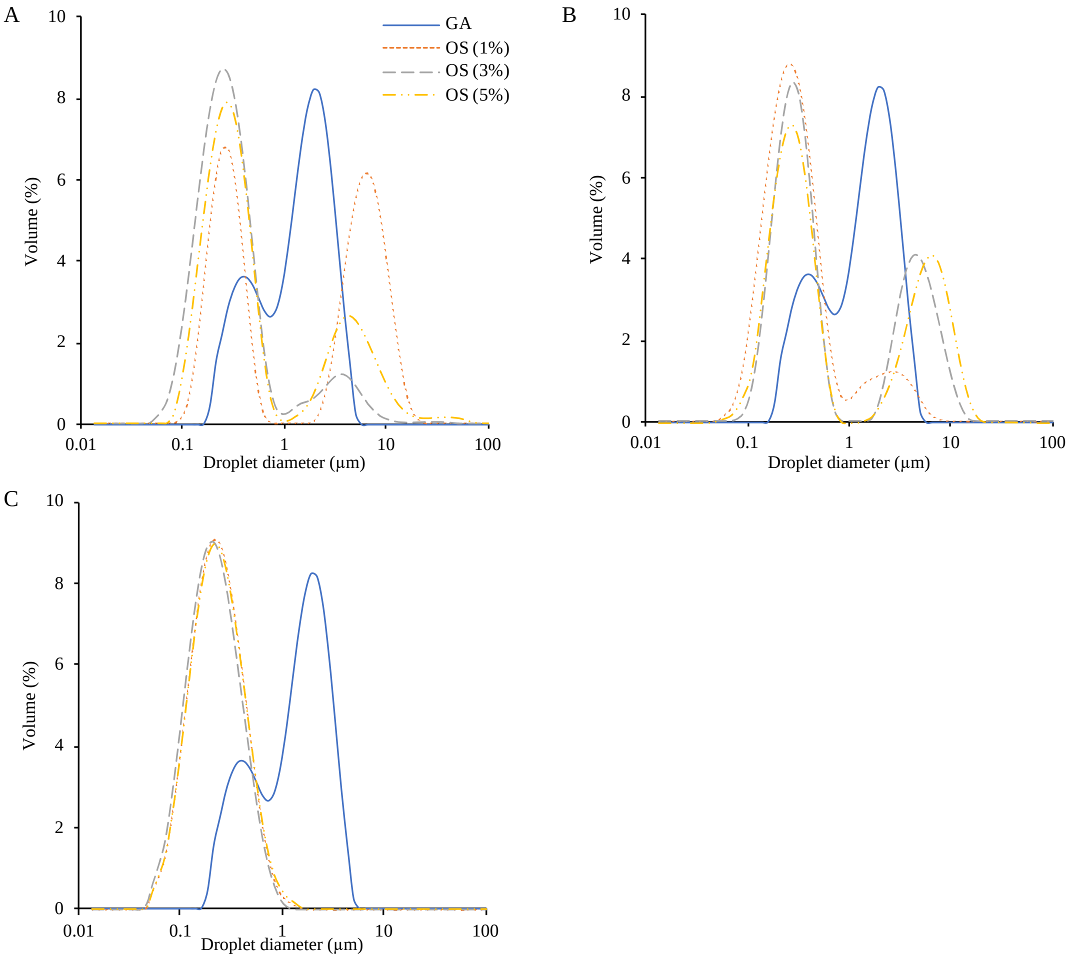


Fig. 4.8. Droplet-size distribution (DSD) curves of emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion) at pH 2. A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS).

4.2.6 Effect of sodium chloride on OS-starch emulsion stability

The $d_{4,3}$ of GA emulsion increased from 0.571 to 1.691 µm when the NaCl concentration in the aqueous medium increased from 0 to 1.0 M (Fig. 4.9. A-1). The zeta potential of GA emulsion was -15.0, -8.1 and -5.8 mV, respectively, when the concentration of NaCl were 0.1, 0.5 and 1.0 M (Fig. 4.9. A-2). The absolute surface charge values of GA emulsion with salt were lower than 30 mV, which could not provide sufficient electrostatic repulsion between the oil droplets (Mirhosseini et al., 2008; Najafi et al., 2016). Therefore, the $d_{4,3}$ of GA emulsion gradually increased as the NaCl concentration was elevated. In addition, the DSD curve of GA emulsion with 0.1 M NaCl showed an obvious shift toward larger diameter, showing a major peak at 0.1-10 µm in Fig. 4.10 (*versus* 0.1-2.5 µm of GA emulsion without NaCl; Fig. 4.4). The changes in the $d_{4,3}$ and the DSD curve of GA emulsion with 0.1 M NaCl were confirmed by the CLSM images in Fig. 4.5. Aggregation of droplets was observed in the image and the diameters of droplets were generally larger than those of the droplets in fresh GA emulsion.

Emulsions prepared with OS PS displayed different extents of destabilization with the existence of NaCl (Fig. 4.9. A-1). The $d_{4,3}$ of the three OS-PS emulsions increased drastically after the addition of NaCl. Generally, the $d_{4,3}$ values of OS-PS emulsions were larger than that of GA emulsion at the same NaCl concentration. Destabilization also occurred to some extents in OS-NCS emulsions with the presence of NaCl as large $d_{4,3}$ values were found, despite insignificant differences being detected among the three NaCl concentrations (Fig. 4.9. B-1). In addition, the $d_{4,3}$ of OS-NCS emulsions were smaller than that of GA emulsion at each NaCl concentration, indicating that OS NCS had stronger ability in stabilizing emulsion than GA under various ionic strength. This might be related to the smaller initial $d_{4,3}$ of OS-NCS emulsions (0.247-0.300 µm) in comparison with that of GA emulsion (0.571 µm). The changes in the $d_{4,3}$ of OS-NCS emulsions due to the presence of NaCl were similar to the findings from previous studies (Guo et al., 2020; Zhao et al., 2017). All OS-PS and OS-NCS emulsions with 0.1 M NaCl showed a bimodal distribution in their DSD curves (Fig. 4.10. A, B): the major peaks of OS-PS emulsions were between 1 and 100 µm, whereas those of OS-NCS emulsions were between 0.1 and 1 µm [except for OS (1%) NCS]. The differences in the DSD curves of the emulsions explained their different $d_{4,3}$ at each NaCl concentration: OS-NCS emulsions < GA emulsion < OS-PS emulsions. Larger droplets and droplet aggregation were observed in the CLSM images of OS PS and OS NCS emulsions with 0.1 M NaCl (Fig. 4.5). OS (1%)-PS emulsion with 0.1 M NaCl consisted of a large proportion of aggregated droplets (indicated by rectangle), different from the uniform and separate droplets found in the fresh emulsion (Fig. 4.5).

Among OS-WCS emulsions, the sample made with OS (1%) WCS became unstable under ionic strength conditions as the $d_{4,3}$ sharply increased with the presence of NaCl (Fig. 4.9. C-1). Bimodal distribution was observed in the DSD curve of OS (1%)-WCS emulsion at 0.1 M NaCl (Fig. 4.10. C). The presence of the peak of 1-10 µm suggested that coalescence occurred in the emulsion, which was confirmed by the CLSM images in Fig. 4.5. In contrast, emulsions formed with OS (3%) and OS (5%) WCS were stable across the NaCl concentration of 0.1-1.0 M as no significant change was detected in their $d_{4,3}$ (Fig. 4.9. C-1). Moreover, the $d_{4,3}$ of the two OS-WCS emulsions were smaller than that of GA emulsion at each salt concentration, indicating the stronger ability of OS WCS than GA in stabilizing O/W emulsion against salt. This finding was supported by the lack of apparent shift in their DSD curves (Fig. 4.10. C) and a similar droplet size in CLSM images (Fig. 4.5) when compared with the fresh emulsion with no NaCl (Fig. 4.4. C-1 and Fig. 4.5). The demonstrated stability of OS (3%) and OS (5%) WCS emulsions against ionic strength in this study is accordance with previous literature (Lin et al., 2018b; Xu et al., 2018).

The magnitude of the negative zeta potentials of the OS-starch emulsions decreased with a higher NaCl concentration, resulting from the electrostatic screening effect of the Na^+ cations (McClements, 2015) (Fig. 4.9. A-2, B-2, C-2). The absolute surface charge values of OS-starch emulsions were reduced to close to zero when NaCl existed in the systems, indicating that electrostatic repulsion among droplets mostly lost under ionic strength conditions. Consequently, the $d_{4,3}$ of the OS-starch emulsions were increased at 0.1 M NaCl. However, no further increment was found in the $d_{4,3}$ of OS-NCS and OS-WCS emulsions when NaCl concentration was elevated to 0.5 and 1.0 M (Fig. 4.9. B-1 and C-1), suggesting that electrostatic repulsion only partially contributed to the stabilization of OS-starch emulsions (Guo et al., 2020).

Generally, the stability of OS-starch emulsions against ionic strength followed an ascending order of OS PS < OS NCS < OS WCS, which could be related to the differences in the steric repulsion of these emulsions as discussed in Section 4.2.5. OS (5%)-PS emulsion exhibit poorest stability upon ionic strength among the three OS-PS emulsions, whereas the three OS-NCS emulsions showed similar stability upon ionic strength. The different behaviors could be possibly related to the difference between the amylose content of PS and NCS (41.5 *vs*. 32.9%, Table 4.1) as amylose tended to affect the steric repulsion in emulsion. Among the emulsions prepared with all the three OS (1%) starches, OS (1%)-NCS emulsion displayed better stability than the other two samples, which could be partly attributed to the higher %OS in OS (1%) NCS (Table 4.2) as OS groups provided steric repulsion to stabilize the emulsion with salt. It is interesting to note that the $d_{4,3}$ of OS-PS and OS (1%)-WCS emulsions decreased as NaCl concentration increased from 0.1 to 1.0 M (Fig. 4.9. A-1, C-1). The phenomenon was probably due to the coalescence of the large droplets in emulsion with higher concentration of NaCl were not detected by the instrument.

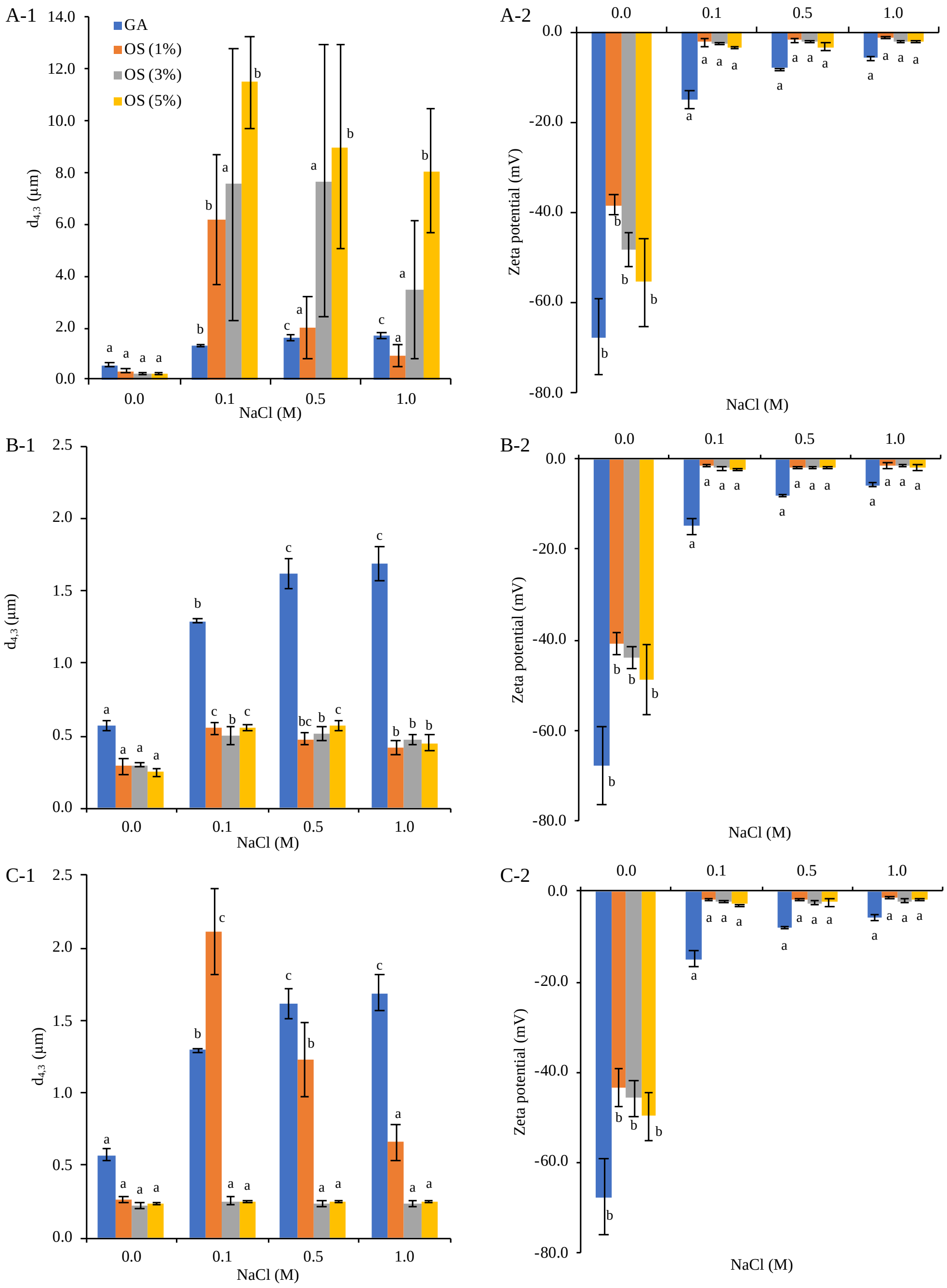


Fig. 4.9. Volume mean droplet diameters ($d_{4,3}$) and zeta potentials of emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion) under the conditions of 0 to 1.0 M NaCl. A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS). The scale of y axis in A-1 was different from those in B-1, C-1. Statistical analysis was conducted among the data obtained from each emulsion under 0 to 1.0 M NaCl in each sub-figure (*e.g.*, A-1, B-1, C-1, A-2, B-2 and C-2). For each emulsion in each sub-figure, data with the same letter are not significantly different at $p < 0.05$.

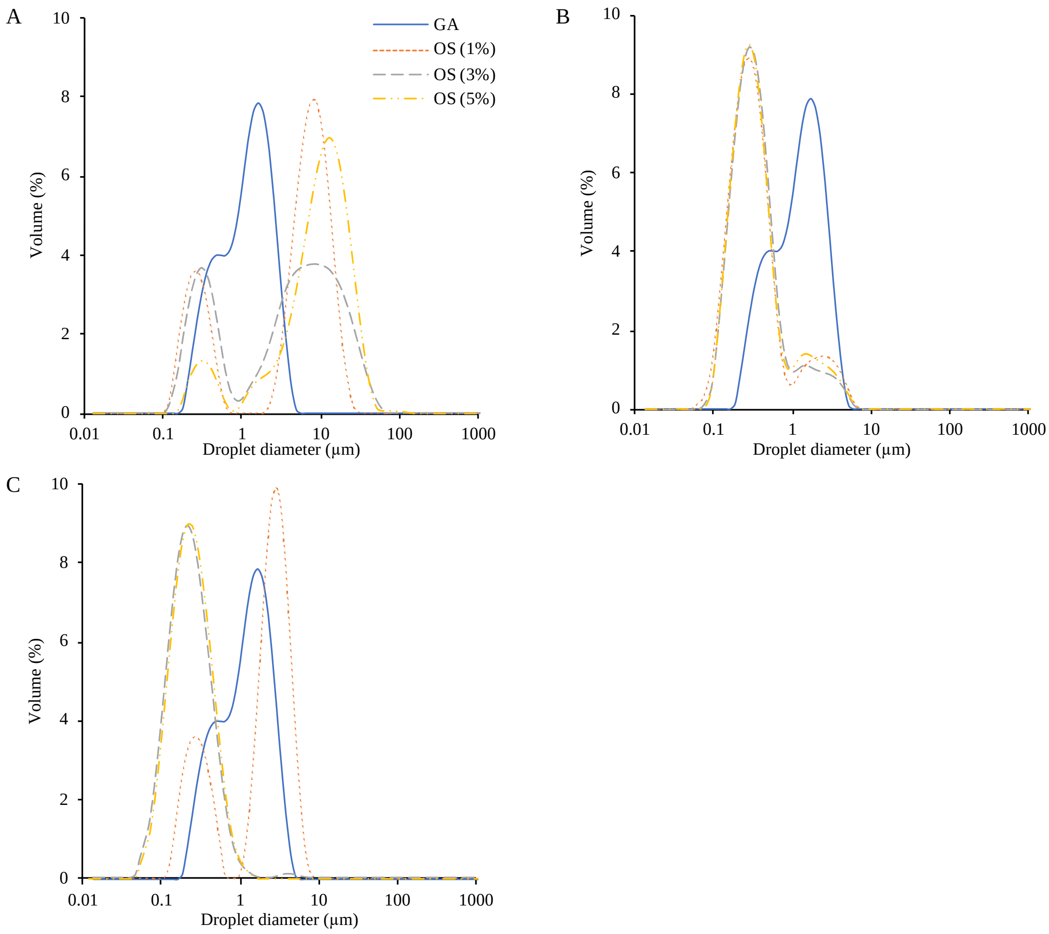


Fig. 4.10. Droplet-size distribution (DSD) curves of emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion) under the presence of 0.1 M NaCl. A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS).

4.2.7 Effect of heating on OS starch stabilized emulsion stability

No apparent changes were found in the $d_{4,3}$, zeta potential, and DSD curve of GA emulsion before and after heating at 90°C for 30 min (Fig. 4.11 and 4.12). These results are in good agreement with those reported by Chanamai and McClements (2002) on the thermal stability of GA emulsion across the temperature range of 30 to 90°C, indicating that GA emulsion was stable upon heating up to 90°C. The CLSM image of GA emulsion after heating confirmed its desirable thermal stability (Fig. 4.5).

By contrast, emulsions stabilized by the OS starches behaved differently after heating in this study. Heating caused destabilization of emulsions developed with OS (1%) PS/NCS/WCS. The $d_{4,3}$ of all the three emulsions at 90°C increased significantly from those of their respective fresh emulsions and became larger than that of GA emulsion at 90°C (Fig. 4.11. A-1, B-1, C-1). After heating, the DSD curves of the three emulsions shifted toward a large diameter value and displayed more than one peak ranging from 0.1 to 100 µm (Fig. 4.12), which explained the marked increase in their $d_{4,3}$.

Emulsions developed with OS (3%) and OS (5%) PS/NCS/ WCS were relatively stable upon heating. No significant increments were observed in their $d_{4,3}$ after heating, despite slight increases being found in the $d_{4,3}$ of OS (3%)-PS, OS (5%)-PS and OS (3%)-NCS emulsions (Fig. 4.11. A-1, B-1, C-1). The DSD curves of these three emulsions exhibited two peaks after heating, with a minor second peak being observed between 1 and 10 µm. Compared with the corresponding fresh emulsions, the DSD curves of OS (5%)-NCS, OS (3%)-WCS and OS (5%)-WCS emulsions showed no obvious change upon 90°C treatment (Fig. 4.12). The changes in the $d_{4,3}$ and DSD curves of the abovementioned emulsions as induced by heating show good consistency with the observation in CLSM images (Fig. 4.5). As examples, a few large droplets were found in OS (5%)-PS emulsion at 90°C; the $d_{4,3}$ of the other emulsions largely remained unchanged after the thermal treatment. The findings are in good accordance with those reported in previous publications, in which incubation at 90°C for 30 min did not induce appreciable change in the mean droplet sizes of the emulsions stabilized by on commercial OS starches (Chanamai & McClements, 2002; Charoen et al., 2011).

The absolute surface charges of the thermally treated OS-starch emulsions showed no change or slight decrease from the respective fresh emulsions at 25°C (Fig. 4.11. A-2, B-2, C-2). The results could partly explain why heating at 90°C did not destabilize the OS-starch emulsions to the same extents as by pH change or the addition of NaCl as discussed above.

Emulsion is a dynamic system, where the oil droplets are moving inside constantly. When emulsion is heated at a high temperature, the provided thermal energy tends to accelerate the movement of droplets. Therefore, the droplets have a higher chance to collide with each other for enhanced aggregation and coalescence (Yan, McClements, Zou, & Liu, 2019). In the current study, OS (1%) starches did not appear to provide sufficient steric and electrostatic repulsion between droplets to stabilize the emulsions against heating. In contrast, OS (3%) and OS (5%) starches, having higher DS (Table 4.2), demonstrated stronger capability of stabilizing the resultant emulsions upon heating. Moreover, induction of more OS groups (higher DS) led to greater binding of OS starch to the oil droplet surface, which resulted in greater emulsion stability against droplet aggregation (Jain et al., 2019).

Overall, the PS, NCS and WCS modified with the same level of OSA showed comparable ability in stabilizing O/W emulsion under heating at 90°C. For all the three types of starches, OS (3%) and OS (5%) starches could better stabilize the emulsion system against thermal treatment up to 90°C when compared with their OS (1%) counterparts.

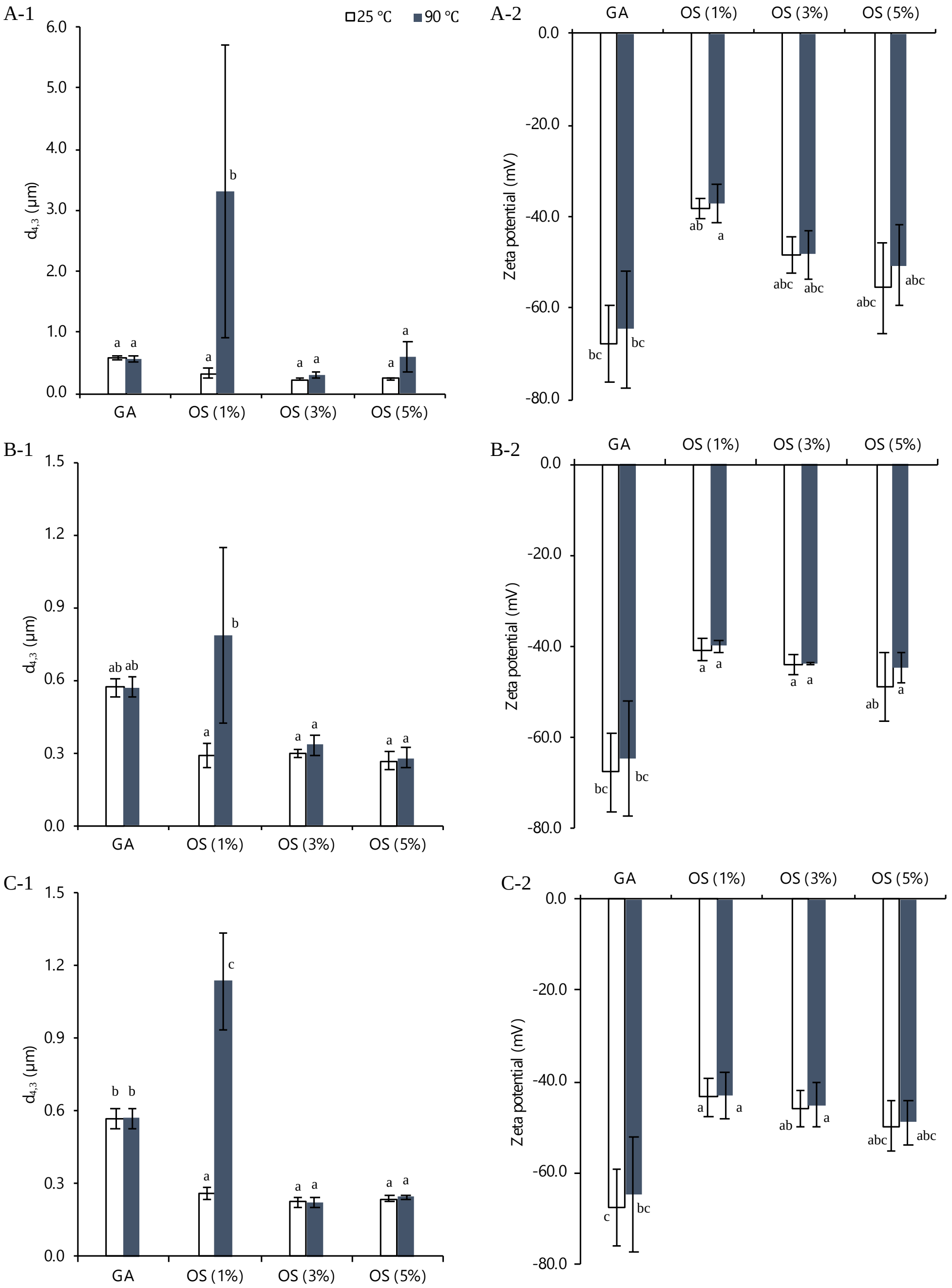


Fig. 4.11. Volume mean droplet diameters ($d_{4,3}$) and zeta potentials of emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion) incubated at 25 and 90°C for 30 min. A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS). The scale of y axis in A-1 was different from those in B-1, C-1. Statistical analysis was conducted among the data obtained from all the eight emulsions (25 and 90°C) in the same sub-figure. In each sub-figure (*e.g.*, A-1, B-1, C-1, A-2, B-2 and C-2), data with the same letter are not significantly different at $p < 0.05$.

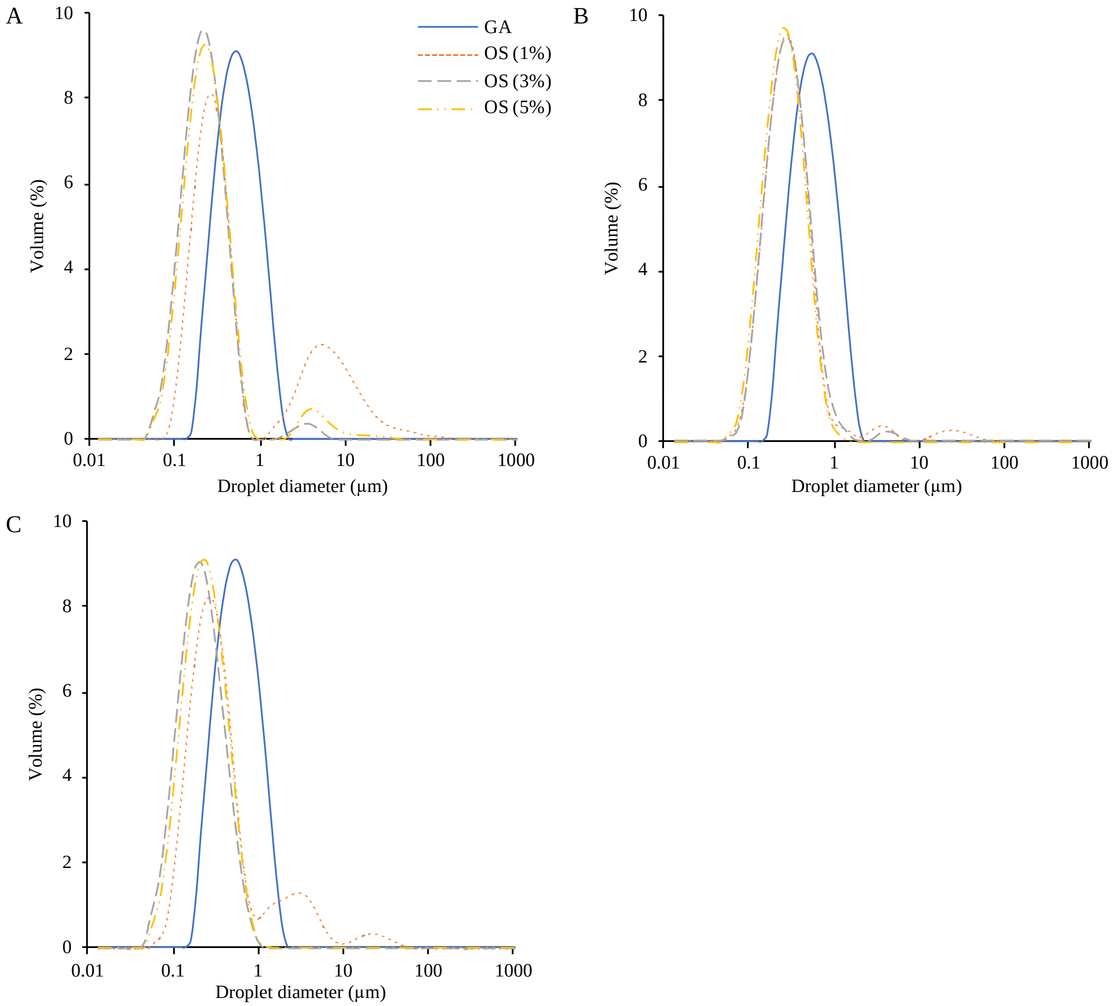


Fig. 4.12. Droplet-size distribution (DSD) curves of emulsions stabilized by gum Arabic (GA, 2 wt%, db, in aqueous dispersion) or OS starches (2 wt%, db, in aqueous dispersion) after incubation at 90°C for 30 min. A: pea starch (PS); B: normal corn starch (NCS); and C: waxy corn starch (WCS).

5. GENERAL DISCUSSION

The objective of this thesis research was to seek the possibility of developing pulse starch emulsifiers using OSA modification, which could potentially enhance the application value of pulse starches. In this study, PS was selected as a representative pulse starch for this purpose, which was compared with commercial NCS and WCS throughout the whole study. The amylose contents of PS, NCS and WCS were 41.5%, 32.9% and 1.7%, respectively (Table 4.1).

In the first part of this thesis, the hypotheses on how the OSA modification would affect the physicochemical properties of the starches were examined. We hypothesized that: (1) OSA modification would markedly change the thermal and pasting properties of PS, NCS and WCS; (2) OS starches would show less tendency to retrograde in comparison with the respective control starches. The three starches were modified with OSA (1%, 3% and 5%, w/w, db), and the %OS, DS, RE, thermal and pasting properties of OS starches were investigated in this part. With an increasing level of added OSA, the DS of the same type of starch gradually increased (Table 4.2). The thermal and pasting properties of the three starches were notably changed after the OSA modification. OS starches showed lower gelatinization temperatures and smaller ΔH when compared with their respective controls, with a more obvious influence being detected in starch modified using a higher concentration of OSA (Table 4.3). The incorporation of OS groups interfered with the re-association between starch chains, and hence the OS starches were found to exhibit less tendency to retrograde (Table 4.3) (Thirathumthavorn & Charoenrein, 2006). Among the three starch types, PS showed the largest percentage of retrogradation at the same level of OSA modification, which was related to the higher amylose content (Table 4.1) and longer amylopectin branch chains of PS (Li et al., 2019). The pasting temperatures of PS, NCS and WCS decreased and their peak viscosities increased after the OSA modification, with a more significant effect being observed for a higher level of OSA used (Fig. 4.1 and Table 4.4). PS showed the lowest peak viscosities among the three starch types for the same concentration of OSA used, which corresponded to the higher amylose content of PS (Table 4.1) as amylose tended to restrict granular swelling of starch during pasting (Li et al., 2019). In general, OSA modification incorporated the negatively charged OS groups onto the starch granules and partly destroyed the starch granular structure, thus contributing to the changes in thermal and pasting properties of the OS starches.

In the second part of the thesis, the emulsifying properties of OS starches and the stability of OS-starch emulsions were evaluated, and GA was included as a commercial standard for comparison. We hypothesized that: (1) OS starch would decrease the oil-water IFT and form O/W emulsion; (2) a higher DS would result in a stronger emulsifying property of the OS starch; (3) the stability of the OS-starch would be independent to 28-d storage, pH changing, ionic strength and thermal treatment. In this part, OS starch was fully gelatinized and dispersed in deionized water prior to the preparation of emulsion. The IFT between canola oil and GA/OS-starch dispersion was determined. All the OS starches exhibited the ability to lower oil-water interfacial tension (Fig. 4.2. A, B, C). The IFT was lowered by OS NCS and OS WCS to the same level as by GA after 2 h equilibrium; however, the values of OS-PS dispersions were larger than those of the other dispersions (Fig. 4.2. D). For the three OS starches from the same origin, insignificant difference was found in the final IFT at 2 h. Emulsions were developed using a high-pressure homogenizer, and the DSD, zeta potential, accelerated stability, and storage stability of the resulting emulsions were characterized and compared. The $d_{4,3}$ of freshly made OS-starch emulsions were smaller than that of fresh GA emulsion (Fig. 4.3. A-1, B-1, C-1). For the fresh emulsions produced with starches modified using the same concentration of OSA, OS-PS emulsion showed a larger $d_{4,3}$ in comparison with those of the OS-NCS and OS-WCS emulsions, with the latter two showing comparable $d_{4,3}$. It is noteworthy that the level of added OSA did not show significant effect on $d_{4,3}$ of the fresh emulsions produced with the three OS starches from the same origin, which is in agreement with the IFT results (Fig. 4.2. D). Due to the existence of OS groups on starch molecules, the surface charge of all the fresh OS-starch emulsions showed a negative value (Fig. 4.3. A-2, B-2, C-2). The electrostatic repulsion among oil droplets could help to stabilize emulsions against storage and changes in environmental conditions.

Accelerated storage stability was determined to assess the long-term stability of the emulsions. The instability indices of OS-starch and GA emulsions were 0.83-0.92 and 0.92, respectively, which indicated that all the emulsions showed similar stability during acceleration (Fig. 4.6. A). The results suggested that the prepared emulsions tended to destabilize, and that phase separation would occur to those systems after being subjected to long-term storage. However, discrepancy was found in the stability data obtained from emulsions that were stored for 28 d. Destabilization only occurred to the emulsions developed with OS PS and OS (1%) NCS/WCS after being stored for 28 d (Fig. 4.3. A-1, B-1, C-1). The discrepant results could be due to the differences in the experimental conditions of the accelerated and 28-d storage tests. Moreover, 28 d might not be sufficiently long to cause destabilization in all the emulsions prepared in the current study. OS-PS emulsions showed different extents of destabilization at Day 28, with a smaller increase in $d_{4,3}$ being observed when DS of the emulsifier was higher (Fig. 4.3. A-1), indicating that a higher DS displayed better capability in stabilizing emulsions upon 28-d storage. In general, the poor storage stability of OS-PS emulsions was related to the greater amylose content (41.5%, Table 4.1) and faster retrogradation rate of OS PS (34.0%-45.7%, Table 4.3). OS (1%) NCS/WCS emulsions destabilized upon 28-d storage as the $d_{4,3}$ was significantly elevated, which was attributed to the lower DS (Table 4.2) and higher percentages of retrogradation (Table 4.3) when compared with their respective counterparts modified using 3% and 5% OSA.

The effects of environmental changes, including pH 2-7, concentrations of NaCl from 0 to 1.0 M and incubation at 90 °C for 30 min, on the emulsion stability were studied as well to predict the behaviour of the prepared emulsions in food products. The emulsion-stabilizing ability of the prepared starch emulsifiers upon changes in pH, ionic strength and storage temperature largely followed an ascending order of OS PS < OS NCS < OS WCS. The absolute surface charge of all the emulsions gradually decreased to a value close to zero due to protonation of carboxyl groups in OS starch with the pH dropping from 7 to 2 (Fig. 4.7. A-2, B-2, C-2). Similarly, when the emulsion systems contained NaCl, the absolute surface charges were reduced to a value close to zero due to the electrostatic screening effect of Na^+ cations (Fig. 4.9. A-1, B-1, C-1). The results of surface charge indicated that the electrostatic repulsion was ineffective in stabilizing emulsions at low pH (4-2) and under strong ionic strength. Therefore, the emulsion stability – particularly with OS WCS – under the examined conditions mainly depended on the steric repulsion generated by the starch layer coating on the surface the oil droplets. The capability of OS WCS in stabilizing emulsions under low pH and strong ionic strength was attributed to the high amylopectin content of OS WCS (98.3% amylopectin, Table 4.1). The highly branched amylopectin molecules provided strong steric repulsion between oil droplets, and the repulsive force could be maintained as it was not easy for the amylopectin molecules to re-associate with each other. In contrast, OS PS and OS NCS provided less steric repulsion to the emulsions due to higher amylose contents

(41.5% and 32.9%, respectively, Table 4.1) than OS WCS (1.7%). In addition, the higher amylose contents of OS PS and OS NCS could facilitate molecular re-association and lead to higher retrogradation rate (Table 4.3). The described physical changes might cause the starch layer on the interface to destabilize, thus weakening the repulsive force. Therefore, OS-PS and OS-NCS emulsions tended to coalesce under low pH (4-2) and ionic strength as both the steric and electrostatic repulsion became weaker upon the changes in the environmental conditions.

Moreover, OS NCS in general displayed better emulsion stabilizing ability than OS PS under the same tested condition. The difference could be attributed to the ~10% difference in the amylose contents of the two starches. Another possible reason for this phenomenon could be the difference in the porosity of PS and NCS granules. The work of Li et al. (2019) revealed that PS granules had a more solid and homogeneous internal structure and that internal voids were observed in NCS granules. Consequently, it might be more challenging for OSA to penetrate into the internal regions of PS in comparison with NCS during starch modification, resulting in less homogenous distribution of OS groups in PS molecules. Thus, OS PS exhibited poorer capability of stabilizing emulsions. For all the three starch types, the samples modified with 3% and 5% OSA generally exhibited stronger ability in stabilizing emulsions upon heating when compared with their counterparts modified with 1% OSA. The differences were attributed to the higher DS of OS (3%) and OS (5%) starches as more OS groups led to better binding of OS starch to the oil-water interface. It is important to note that OS starch showed advantages in keeping emulsion stable upon low pH (4-2), ionic strength, and high temperature in comparison with protein, which is another category of emulsifier for food use (Chanamai & McClements, 2002; Charoen et al., 2011). The indicated conditions can easily alter the structure of protein molecules, which could be detrimental for them to function as an effective emulsifier to stabilize the system.

Overall, OS PS generally displayed poorer emulsifying property and emulsion stabilizing ability than OS NCS and OS WCS at the same level of OSA modification. However, OS (3%) PS and OS (5%) PS showed the potential to be utilized as effective emulsifiers for different applications.

6. SUMMARY AND CONCLUSIONS

In the current study, PS (41.5% amylose), together with NCS (32.9% amylose) and WCS (1.7% amylose), was modified using 1%, 3% and 5% OSA. %OS and DS of the OS starches from the same starch sample progressively increased as more OSA was used for the modification. The thermal and pasting properties of the three starches were significantly altered by OSA modification. As the DS increased, gelatinization temperatures and enthalpy changes of the OS starches gradually decreased, and the OS starches displayed a less tendency to retrograde. OSA modification noticeably reduced the pasting temperatures and increased the peak viscosities of the starches, with more obvious effect being noted with a higher DS as well.

The OS starches presented the ability to lower oil-water IFT, which value gradually decreased as a function of time. O/W emulsions were produced with the derived OS starches, and the resultant fresh emulsions showed $d_{4,3}$ smaller than that of the emulsion prepared with GA. The OS-starch emulsions showed better stability than GA emulsion under accelerating condition as the creaming velocities of droplets in the former group were noticeably slower than that of the latter, which could be explained by the markedly smaller $d_{4,3}$ and higher viscosity of OS-starch emulsions.

After 28-d storage, the $d_{4,3}$ of OS-PS emulsions increased to different extents, which were larger or comparable to that of GA emulsion on Day 28. A smaller extent of increment in $d_{4,3}$ of OS-PS emulsions was observed as the DS increased. OS-NCS and OS-WCS emulsions generally displayed better stability than GA emulsion upon 28-d storage as destabilization was only observed in emulsions developed with 1% OSA modified starches. The destabilization was attributed to smaller DS and higher retrogradation percentage of OS (1%) NCS/WCS. The $d_{4,3}$ of all OS-NCS and OS-WCS emulsions on day 28 remained smaller than that of GA emulsion. Overall, OS PS exhibited poorer ability to stabilize O/W emulsion, which could be partly explained by that the stronger re-association and a higher rate of retrogradation between starch molecules in the emulsifier layer reduced the steric hindrance between droplets.

The emulsions developed with OS (1%) starches generally showed poor stability against various environmental changes. By contrast, the emulsions prepared with the starches modified with 3% and 5% OSA were relatively stable and displayed droplet size comparable or smaller than that of GA emulsion after being subjected to pH 4-7 and incubation at 90°C for 30 min. However, obvious aggregation and coalescence occurred in OS-PS emulsions when the systems contained NaCl, with $d_{4,3}$ larger than that of GA emulsion at each concentration of NaCl. OS-NCS emulsions displayed stronger stability than GA emulsion under ionic strength, with only slightly destabilization being detected in the former. It was interesting to note that WCS modified with 3% and 5% OSA showed strong ability to keep the emulsions stable upon all the tested environmental conditions, which had $d_{4,3}$ smaller than that of GA under the same condition. The examination of the $d_{4,3}$ and zeta potentials of all the OS-starch and GA emulsions subjected to different environmental changes indicated that the primary mechanism for OS starches and GA to stabilize O/W emulsion was steric repulsion and that electrostatic repulsion only partly contributed to the stability of such an emulsion system.

Overall, the emulsion stabilizing ability of the investigated emulsifiers upon 28-d storage, 0-1.0 M NaCl, and incubation at 90 ºC for 30 min followed an ascending order of OS PS < GA ≤ OS NCS < OS WCS. The emulsion stabilizing ability of OS starches was affected by their DS and structure. For the same starch type, OS starch with a higher DS tended to exhibit better capacity of stabilizing emulsion against 28-d storage and changes in environmental conditions. This was due to the fact that OS groups contributed to the steric hindrance and electrostatic repulsion between emulsion droplets. The higher amylose content and longer amylopectin branch chains (Li et al., 2019) of PS resulted in stronger molecular reassociation and higher rate of retrogradation in OS PS. Consequently, the OS PS layer coating on the droplets tended to destabilize upon the physical changes and to weaken the steric hindrance in OS-PS emulsions, leading to poorer emulsion stabilizing behavior of OS PS than the other OS starches. In contrast, the amylopectin of WCS (98.3% of the starch) contributed to strong steric repulsion in OS-WCS emulsions, and the repulsion force could be better retained due to less molecular re-association and retrogradation. Therefore, the OS-WCS emulsions generally exhibited desirable stability upon 28-d storage and different environmental conditions.

The results obtained from this thesis largely proved the hypotheses proposed in Section 1.3. The thermal and pasting properties of PS, NCS and WCS were significantly altered by OSA modification, with a more obvious effect being detected at a higher level of used OSA. OS starches exhibited ability in reducing oil-water IFT and creating O/W emulsions. Emulsifying properties of OS starches and stability of the resultant emulsions followed the ascending order of OS PS < OS NCS < OS WCS, and higher DS tended to enhance the emulsifying property of the OS starch as well as the stability of OS-starch emulsion.

In general, OS PS displayed poorest capability in stabilizing O/W emulsions upon storage and change of environmental conditions among three starches. The results suggested that OS PS might not be suitable to be used as commercial emulsifiers in comparison with GA, OS NCS and OS WCS. However, the results from this thesis still indicated the possibility of using OS (3%) and OS (5%) PS as an emulsifier in certain O/W emulsions,. The formed emulsions mostly remained stable after 28-d storage at 4°C, over a pH range of 4-7, and upon heating at 90°C for 30 min. The acquired new knowledge can be applied to diversify the functional properties of pea and other pulse starches. The advanced understanding of how the structural features of OS starches influenced the emulsifying properties can be utilized to design the structure of starch molecules that will be more suitable for emulsion applications.

7. FUTURE STUDIES

In the current thesis, PS, NCS and WCS were modified using OSA to prepare starch-based emulsifiers. The time that was required to complete the modification varied among the three starches. For example, during the modification of pea starch with 5% OSA, the whole process lasted for approximately 5 h; while the modification of the two corn starches at 1-5% OSA only consumed around 2.5 h. Future studies can be conducted to optimize the modification conditions for PS (possibly other pulse starches) to shorten the modification time, while maintaining or even improving the DS level.

According to collected data, the stability of the O/W emulsions prepared with OS PS was not comparable to that of the OS-WCS emulsions, which could be mainly attributed to the higher amylose content of the former (41.5% *vs.* 1.7%). A lower amylose content in OS starch is more favorable for the formation of O/W emulsions that are stable toward storage and environmental changes in the systems (*e.g.*, pH, ionic strength and temperature) (Altuna et al., 2018). Therefore, it is meaningful to use some modification methods to alter the structure of native PS or OS PS to further enhance the performance of final OS PS product as an emulsifier, for instance, decreasing the amylose content of starch. Hydrolysis by acid or amylolytic enzymes can be employed to achieve this goal as the two approaches degrade starch molecules and hence change the amylose content of starch. For example, β-amylolysis has been adopted as a dual modification approach to further tailor the structure of OS WCS, which significantly improved its emulsifying capability (Xu, Huang, Fu, & Jane, 2015). It will be worthwhile to explore other dual modification methods to prepare emulsifiers from PS with more desirable functional attributes.

The main objective of the current study was to evaluate the emulsifying capability of OS starch in O/W emulsion. Nonetheless, it will be meaningful to explore the potential of utilizing OS PS in other emulsion systems, such as Pickering emulsion. OSA modification is also an effective method that can be used to increase resistant starch content of starch ingredients (Ai et al., 2013). *In vitro* and *in vivo* digestibility of OS PS can be determined to reveal whether this modified starch can be used as a functional ingredient to enhance the carbohydrate nutritional value of food products. Moreover, the *in vitro* digestibility of OS-PS emulsion can be examined to illustrate the potential of utilizing OS PS to enhance the bioavailability of bioactive compounds (*e.g.*, β-carotene). Studies related to encapsulation and drug delivery using OS starch stabilized emulsion systems can be conducted in the future as well.

In the experimental design of this study, only the effect of individual parameter of the environmental conditions (*i.e.*, pH, salt or temperature) on the stability of the prepared emulsions was evaluated. However, during food processing, O/W emulsions may be subjected to the changes in various conditions simultaneously. Therefore, the stability of the resultant emulsions can be determined under more complicated conditions, which will better reflect their performance in real food systems (Zhao et al., 2017).

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9. APPENDICES

9.1 List of table

Table A1 Chemical compositions of pea starch (PS), normal corn (NCS) and waxy corn starches (WCS) used in the current study[a].

	Starch content (%, dry basis)	Protein content (%, dry basis)	Ash content (%, dry basis)
PS	94.8	0.2	0.03
NCS	95.8	0.3	0.08
WCS	97.5	0.1	0.15

[a] Adapted from Li et al. (2019).

9.2 List of figures

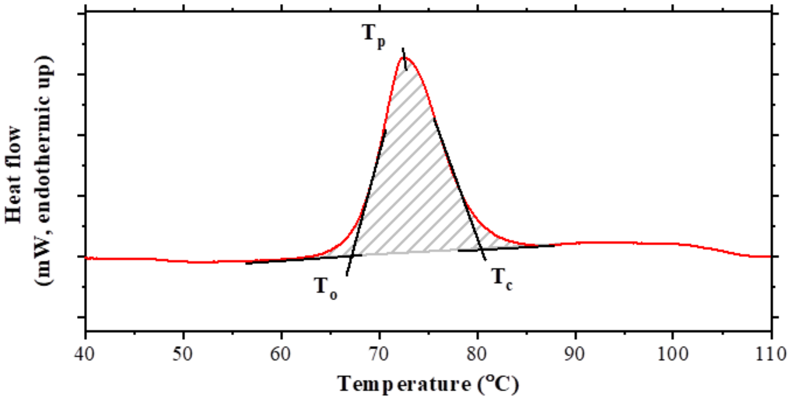

Fig. A1 Differential scanning calorimetry thermogram of normal corn starch modified with 1% OSA. Enthalpy change (ΔH) of starch gelatinization is calculated as: $\Delta H = \frac{A \times 60 \times B \times E \times \Delta qs}{M \times C}$, where A is the area under the curve (inch^2); M is the sample mass (mg); C is the sample concentration (%, w/w); B is the time base setting (min/inch); E is the cell calibration coefficient (no unit); and Δqs is the y-axis range (mJ/sec/inch) (Biliaderis, Maurice & Vose, 1980).

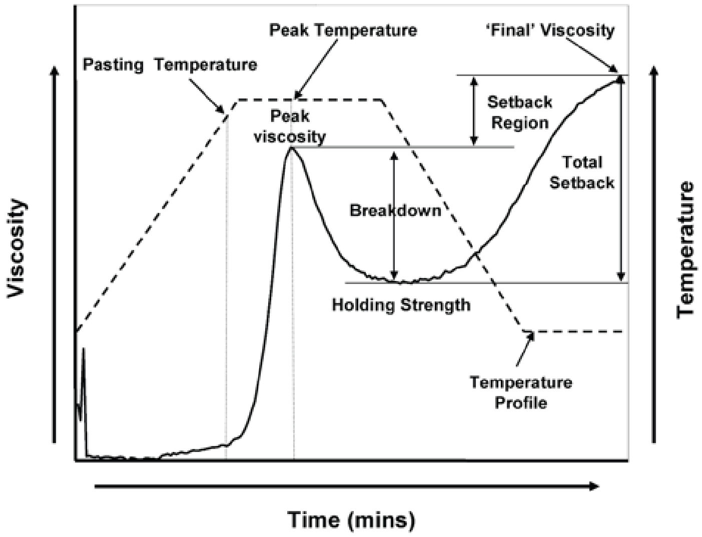

Fig. A2 Typical starch pasting curve showing pasting parameters measured with Rapid Visco Analyser or Amylograph [with permission from Wani et al., (2012)].

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