Carbohydrate Polymers xxx (xxxx) xxx-xxx

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: http://ees.elsevier.com

Physicochemical and functional properties of native and modified agave fructans by acylation

A. Ignot-Gutiérrez [a], R.I. Ortiz-Basurto [b], O. García-Barradas [c], D.I. Díaz-Ramos [b], M. Jiménez-Fernández [d,*]

[a] Maestría en Ciencias Alimentarias, Universidad Veracruzana, Xalapa, Veracruz, Mexico
[b] Lab. Integral de Investigación en Alimentos, TecNM-Instituto Tecnológico de Tepic, Tepic, Nayarit, Mexico
[c] Unidad de Servicios de Apoyo en Resolución Analítica, Universidad Veracruzana, Xalapa, Veracruz, Mexico
[d] Centro de Investigación y Desarrollo en Alimentos, Universidad Veracruzana, Xalapa, Veracruz, Mexico

UNCORRECTED PROOF

ARTICLE INFO

Chemical compounds studied in this article
D-Fructose (PubChem CID: 5984)
Lauroyl chloride (PubChem CID: 8166)
Sodium hydroxide (PubChem CID: 14798)
Hexane (PubChem CID: 8058)
Deuterated water (PubChem CID: 24602)
Deutero chloroform (PubChem CID: 71583)
Phosphate buffered saline (PubChem CID: 24978514)
Nitrogen gas (PubChem CID: 947)
Lauric acid (PubChem CID: 3893)

Keywords
Fructans
Acylation
Esterification
Modified carbohydrates
Surface tension

ABSTRACT

Native agave fructans were modified by an acylation reaction with lauric acid. Native and modified fructans were characterized using NMR, FTIR and various physicochemical and functional properties at different pHs were evaluated. NMR and FTIR spectra demonstrated the incorporation of lauric acid in the molecular structure of fructans. Modified agave fructans exhibited a color, moisture and water activity similar to native fructans, but properties such as solubility, swelling capacity, emulsifying activity and foam capacity were significantly modified by the acylation reaction mainly when the samples were analyzed at different pHs. The thermogram of the acylated fructans evidenced significant changes in thermal properties when compared with native fructans and acylated fructans were able to form micellar aggregates. In general, modified fructans showed improved functional properties in comparison with native fructans representing an important opportunity to improve the functionality of the foods in which it is incorporated.

1. Introduction

Fructans are a mixture of fructooligosaccharides and fructose-fructose polymers linked by glucosidic bonds β (2-1) and β (2-6) (Toriz, Delgado, & Zuñiga, 2007), whose molecular structure varies depending on the source of extraction, conditions of cultivation, soil and climate (Mancilla-Margalli & López, 2006). Its use has expanded to various foods and additives due to its many proven properties, such as prebiotic potential, in aspects of metabolic, immunity and toxicological syndrome and as encapsulating materials, since they have low calorie sweetening properties, among others (Ortiz- Basurto, Rubio Ibarra, Ragazzo Sanchez, Beristain, & Jiménez Fernández, 2017; Urías-Silvas et al., 2008; Márquez-Aguirre et al., 2013). For this reason, its use and incorporation in various food and pharmaceutical products has been increased and encouraged. Fructans are currently used because of their functional properties as fat and sugar substitutes and have been added to yogurt, ice cream, cookies, bread, and various dairy products (Akin, Akin, & Kirmaci, 2007; Pintor-Jardines et al., 2018; Santiago-García, Mellado-Mojica, León-Martínez, & Lopez, 2017). They have also been used as wall materials in the encapsulation of antioxidants and as adjuvants in the pharmaceutical industry (Espinoza-Andrews & Urías-Silvas, 2012). However, it has been shown that these have a high solubility and hygroscopicity, since due to the amount of simple sugars present they have a low glass transition temperature in the range of 5–68 °C (Espinoza-Andrews & Urías-Silvas, 2012; Raghavendra, Rastogi, Raghavarao, & Tharanathan, 2004), which causes the stickiness and solubility of the materials to which it is added to be promoted at room temperature limiting its use in certain foods (Zotarelli, Martins-Da Silva, Durigon, Dupas-Hubinger, & Borges-Laurindo, 2017; Ortiz- Basurto et al., 2017). So, the challenge is to modify its structure for diversify and expand the use of agave fructans in foods without compromising their functional properties. For this reason, several chemical, physical and enzymatic treatments have been tested to modify properties (Mira-

* Corresponding author.
E-mail address: maribjimenez@uv.mx (M. Jiménez-Fernández)

https://doi.org/10.1016/j.carbpol.2020.116529
Received 24 January 2020; Received in revised form 18 April 2020; Accepted 27 May 2020
Available online xxx
0144-8617/© 2020

montes-Corona et al., 2020). Fructans have hydroxy groups in C3, C4 and C6 carbons per unit of fructose, which provides active sites for esterification (Miramontes-Corona et al., 2019). This allows fatty acids to be incorporated into their chemical structure, which favors the production of improved properties (Casas-Godoy, Arrizon, Arrieta-Baez, Plou, & Sandoval, 2016). The chemical modification of inulin-type polysaccharides has been studied to obtain hydrophobic and water-resistant products by means of a chemical reaction (Namazi, Fathi, & Dadkhah, 2011). It has been reported that due to the hydrophilic nature of fructans, their modification with hydrophobic groups is necessary, so that the insertion of groups such as lauric acid allow greater compatibility and better encapsulation of hydrophobic drugs (Kalepu & Nekkanti, 2015; Rogge, Stevens, Colpaert, Levecke, & Booten, 2007). It is important to emphasize that the incorporation of hydrocarbon chains such as lauric acid might modify the hydrophilicity of fructans confering them amphiphilic properties and favoring their use as stabilizers in emulsions or liquid dispersions (Morros, Levecke, & Infante, 2010). Which, does not represent any risk for the human organism since the carbohydrate-fatty acid complex can be synthesized and metabolized by the human organism (Horton, Goldstein, & Brown, 2002). In addition, it has been reported that the hydrophobic characteristics of the agave fructans represent a great potential for the release of drugs in the colon, favoring their permanence for a longer time and ensuring their delivery and release (Miramontes-Corona et al., 2020). On the other hand, the physicochemical and functional properties of biopolymers are strongly dependent on the pH to which they are exposed (Cerdan-Leal, Lopez-Alarcon, Ortiz-Basurto, Luna-Solano, & Jimenez-Fernandez, 2020), so the evaluation of the acylated fructans to different pH will generate knowledge that will help diversify their use of different products. The hypothesis of this work is that the modified fructans by acylation reaction improve physicochemical and functional properties proposing them for use and incorporation in several food and pharmaceutical products. For this reason, the objective of the work was to evaluate the physicochemical and functional properties at different pHs of the native agave fructans and modified by an acylation reaction with lauric acid.

2. Materials and methods

2.1. Material

Native agave fructans with a maximum degree of polymerization around 70 (molecular weight of around 11 kDa) were donated by the company Mieles Campos Azules S.A. of C.V. Lauroyl chloride (C12) and all reagents were obtained from Aldrich Chemical Co.

2.2. Acylation of native fructans

The acylation of the native agave fructans was performed according to the methodology reported by Han, Ratcliffe, and Williams (2017) with some modifications. First, 10 g of native fructans and 50 mL of 20 % w / v NaOH were placed in a glass of precipitate while stirring at room temperature until complete dissolution. Subsequently, 1.5 mL of lauroyl chloride was added dropwise. The reaction could continue for 1.5 h to ensure complete replacement of the compound. The solid product obtained was recovered by filtration and wash with hexane using soxhlet extraction to remove the unreacted fatty acids. Finally, the product was dried in an oven at 45 °C for 48 h.

2.3. Characterization of native and modified fructans

2.3.1. ^{1}H NMR spectroscopy

^{1}H NMR spectra were performed for native and acylated agave fructans on a Nuclear magnetic resonance spectrometer (Agilent Technologies, Model DD2 500 MHz, USA). 16 mg of sample was used and 500 μL of D_2O was used as solvent (4.8 ppm) for native fructans and $CDCl_3$ (7.25 ppm) for acylated fructans. Spectral analysis and display were performed using MestReNova. Mestrelab Research. Chemistry Software Solutions. Versión 6.0.2–5475. The degree of substitution of the acylated fructans was obtained according to the methodology reported by Miramontes-Corona et al. (2020).

2.3.2. Fourier-transform infrared spectroscopy (FTIR)

Native and acylated agave fructans were analyzed fourier transform infrared spectroscopy (FTIR, Agilent Technologies Cary 600, USA) in transmission mode. The samples (2 mg) were placed directly in the equipment without prior preparation. Transmission spectra were recorded using a resolution of 8 cm^{-1} in the spectral range 4000–400 cm-1. Spectral analysis was performed Resolutions Pro. FTIR Spectroscopy Software. Version 5.3.0.1694 (Okunlola, Sarafadeen, & Adeyeye, 2017).

2.3.3. Thermal properties

The thermal properties of native and acylated agave fructans were performed using Differential Scanning Calorimetry equipment (DSC) (TA instruments, USA) under a nitrogen atmosphere. Each sample was heated from 0 to 200 °C at a rate of 5 °C / min. A double empty capsule was used as a reference. All determinations were developed in triplicate. The DSC equipment was preliminarily calibrated with a standard reference of indium.

2.4. Physicochemical properties of native and modified fructans

The moisture content of the native and modified fructans was determined gravimetrically by vaccum drying oven at 70 °C for 24 h until the samples reached a constant weight (AOAC, 1990). The water activity was measured at 25 °C using an Aqualab hygrometer model Series 3, Decagon Devices Inc., Pullman, WA, USA. The color of the samples was evaluated using a spectrophotometer (Color Flex model Hunter Lab CX115 45/0, Reston VA, USA) through the parameters L *, a *, and b * by CIE Lab scale and the Hue angle and Chroma were calculated.

2.4.1. Surface tension

The surface tension at the air / water interface was measured at concentrations between 0–1 mg / mL at 25 ± 1 °C using distilled water as a solvent. A tensiometer (DCAT brand model 11) was used by the Wilhelmy plate method. The Wilhelmy plate used was 10 mm long, 19.9 mm wide, and 0.2 mm high (Kokubun, Ratcliffe, & Williams, 2013). The Critical Aggregation Concentration (CAC) was determined from the change in slope of the plot of equilibrium surface tension as a function of concentration.

2.5. Water holding capacity (WHC) and oil holding capacity (OHC)

Water and oil holding capacity were determined by the method of Wani, Sogi, Wani, & Gill (2013). A dispersion of 0.25 g of sample was dispersed for 1 min in 2.5 mL of water or pure soybean oil for the determination of water or oil retention capacity, respectively. Samples were allowed to settle for 30 min and centrifuged (Eppendorf brand, model 5804 R, Germany) at 2199 g for 10 min. The water or oil released in the centrifugation was discarded and the tubes were weighed. The water or oil holding capacity was expressed in g of water or oil retained per g of sample.

2.6. Functional properties of native and modified fructans

2.6.1. Solubility Index (SI)

The solubility of the native or acylated agave fructans was determined according to the method reported by Acosta-Domínguez, Hernández-Sánchez, Gutiérrez-López, Alamilla-Beltrán, and Azuara (2016) with some modifications. A dispersion of the sample at 1 % (w/v) was prepared in 0.1 M phosphate buffer a pH 4, 7 and

UNCORRECTED PROOF

10. The dispersions were stirred for 30 min and centrifuged in centrifuge (Eppendorf brand, model 5804 R, Germany) at 3550 g for 20 min at 20 °C. The remaining sediment was collected and dried at 100 °C for 30 min.

2.6.2. Swelling capacity

The swelling capacity was determined according to the method described by Gómez-Ordóñez, Jiménez-Escrig, and Rupérez (2010), in which, 250 mg of dry sample were placed in graduated tubes of 10 mL and 5 mL of phosphate buffer was added at pH 4, 7 and 10, respectively. It was shaken gently to remove entrapped air bubbles and the tubes were placed on a level surface, allowed to settle at room temperature for 24 h. The volume in mL occupied by the sample was measured and the swelling capacity was reported as mL / g dry sample.

2.6.3. Emulsifying capacity

The emulsifying capacity was determined by the method described by Acosta-Domínguez et al. (2016) with some modifications. To determine the emulsifying capacity, suspensions of samples at 4 % were prepared in phosphate buffer at pH 4, 7 and 10 in graduated centrifuge tubes. 5 mL of soybean oil were added and homogenized at 10,000 rpm for 1 min in a homogenizer (Wiggen Hauser, model D-130, Malaysia). Then, tubes were centrifuged at 2029 g for 5 min at 20 °C. The emulsifying activity was calculated according on the following equation:

$$\text{Emulsifying Capacity} = \frac{\text{Height of emulsionated layer}}{\text{Total height}} * 100 \quad (1)$$

2.6.4. Foaming capacity and stability

The foaming capacity was determined by the method of Wani et al. (2013). Aqueous dispersions at 2 % w / v of the sample at pH 4, 7 and 10 was prepared and homogenized at 10,000 rpm for 1 min in a homogenizer (Wiggen Hauser, model D-130, Malaysia). The foaming capacity was calculated as the percentage increase in volume of the sample dispersion. Foam stability was determined as the time in minutes it took for the foam to return to the original volume.

2.6.5. Zeta potential

The zeta potential of the particles of the dispersed native and acylated agave fructans (1 %) was evaluated at pH 4, 7 and 10 at 25 °C, according to the method reported by Mendoza-Sánchez et al. (2018) using a Zetasizer Nano ZS device (Malvern Instruments Ltd., Worcester, UK) applying an electric field with a voltage of 150 V.

2.7. Statistical analysis

For the statistical analysis, the statistical package Minitab Graphs version 16 was used and a Multivariate Factorial ANOVA was used for the comparisons. Averages analysis was also performed using the Tukey test with $\alpha = 0.05$.

3. Results and discussion

3.1. Characterization of native and modified fructans

3.1.1. Characterization by ^{1}H NMR

The acylation reaction was corroborated by ^{1}H NMR. Fig. 1 shows the spectrum of native agave fructans in which the signal of the —OH groups of the fructose structure at 3.25 ppm and 4.25 ppm can be identified, coinciding with that reported by Han et al. (2017). In contrast, acylated agave fructans showed a spectrum with absence of the signal from the OH groups, indicating the complete replacement of the —OH groups present in the native fructans with lauric acid. The above was corroborated, since new signals at 0.8 ppm and 1.25 ppm are characteristic of the presence of CH_3 and CH_2, respectively, corresponding to lauric acid (Namazi et al., 2011). On the other hand, the degree

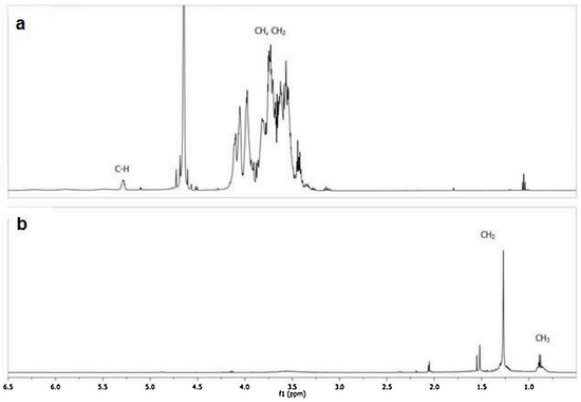

Fig. 1. ^{1} H NMR (a) spectra of native agave fructans and (b) modified agave fructans. The 4.8 ppm signal in Figure (a) is indicative of D_2O used as a solvent.

of substitution of the acylated fructans was 2.34, which is below the maximum percentage of substitution allowed for acylated starches according to the Food Additives and Contaminants Committee Report on modified starches (FAC (Food additives & contaminants committee report on modified starches), 1980), and this value is similar to the degree of substitution reported for acetylated fructans (Miramontes-Corona et al., 2020).

3.1.2. Characterization by FTIR

FTIR spectra confirmed the replacement of the hydroxyl groups of native agave fructans with lauroyl chloride (Fig. 2). In the FTIR spectra they showed absorption bands in 3290, 2928, 2360, 1961, 1648, 1416, 1320, 1121, 1015, 925 cm^{-1} characteristics of native agave fructans. The 3290 cm^{-1} peak indicates the stretching of the hydroxyl groups (OH) while the 2928 cm^{-1} peak is attributed to the extension of C—H. On the other hand, the peak of 1416 cm^{-1} is associated with CH_2—OH of the fructose ring and the peaks between 1320 and 925 cm^{-1} are typical of carbohydrate structures with COC bonds between the monomers that make it up (Apolinário et al., 2017). In turn, the spectrum corresponding to acylated agave fructans showed a significant decrease in the 3290 cm^{-1} peak bands attributed to the OH groups and the 1320 and 925 cm^{-1} peak bands attributed to the bonds COC, suggesting the replacement of the OH groups of native fructans. Similarly, the appearance of two peaks was observed in 1556 and 1421 cm^{-1}, which are absent in the spectrum of native fructans and that are related to the stretching of the carbonyl group present in the ester resulting from the acylation reaction. Similarly, the 2847 cm^{-1} peak is related to aliphatic C–H stretches, and the 2921 cm^{-1} peak increase is associated with grafting of CH_3 and CH_2 groups. These signals are similar to those reported by the addition of fatty acid to starches (Grube, Beckers, Upite, & Kaminska, 2002; Namazi et al., 2011).

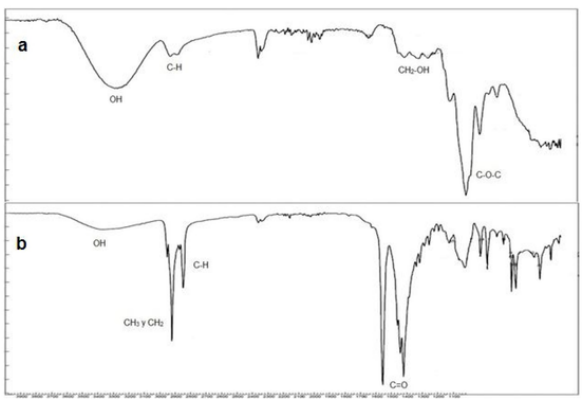

Fig. 2. FTIR spectra of native agave fructans (a) and acylated agave fructans (b).

INCORRECTED PROOF

Although there are several published works of modification of fructans (Miramontes-Corona et al., 2019, 2020) to date none has addressed the safe use of modified agave fructans. However, the acylation of starches with fatty acids has been used in several pharmaceutical and food products considering them as safe ingredients (FAC (Food additives & contaminants committee report on modified starches), 1980). Some of them have been used as encapsulating materials, packaging or as food additives. However, it is highly recommended to perform *in vivo* tests before using agave fructans as a food additive.

3.1.3. Thermal analysis of fructans

The thermal transitions of native and modified fructans were analyzed by Differential Scanning Calorimetry (DSC) (Fig. 3). The thermogram of native fructans shows a single endothermic peak between 119–160 °C (ΔH = 22.45 J / g), which is attributed to the melting point. Similar results were reported for agave fructans and attributed to relaxation enthalpy energy on the amorphous polymers chains (Espinoza-Andrews & Urías-Silvas, 2012). The thermogram of the acylation modified agave fructans showed two endothermic peaks, the first at 72.83 °C (ΔH = 4.158 J / g) and one greater at 106–160 °C (ΔH = 79.82 J / g). This difference in thermograms may be related to the incorporation of lauric acid in the structure of fructans, which possibly produce a change in the conformation or molecular rearrangement of the terminal chains in which the hydroxyl groups have reacted with the lauroyl chloride by modifying its molecular mobility (Moreno-Vilet et al., 2014). It is important to consider that agave fructans are a complex mixture of carbohydrates that have different structures and polymerization degrees (Mancilla-Margalli & López, 2006) and possibly the lauric acid added to the structure of fructans produces an interference with the hydrogen bonds weaken its structure and decrease in the gelatinization temperature (Singh, Kaur, & McCarthy, 2007; Yang et al., 2016). On the other hand, the glass transition temperature (Tg) is considered responsible for the viscoelastic properties of fructans. The thermogram shows a glass transition temperature for unmodified fructans in a range between 45–55 °C, which coincides with the Tg reported for inulin (Apolinário et al., 2017), while modified fructans have a Tg at 25–35 °C. These values are different from what was reported by Miramontes-Corona et al. (2020). These differences could be due to the moisture content of the sample, the molecular weight, its crystallinity (Espinoza-Andrews & Urías-Silvas, 2012), or the degree of polymerization since the presence of monosaccharides (especially fructose) has been reported to have an negative impact on Tg (Kawai, Fukami, Thanatuksorn, Viriyarattansak, & Kajiwara, 2011), which should be considered for the subsequent application of modified fructans.

3.1.4. Surface tension and critical micellar concentration of fructans

The surface tension of native and modified fructans was plotted as a function of concentration. Fig. 4 shows that the surface tension for native fructans did not change significantly in the range of concentrations analyzed. This was possibly due to the high number of hydroxyl groups present in the fructan molecule, which cause high water solubility (Cruz-Cardenas et al., 2015). In contrast, acylated fructans at a concentration of 0.5 mg / mL had an inflection in surface tension (50 mN / m), which corresponds to the critical micellar concentration, indicating that micellar aggregates formed in the aqueous suspension of acylated fructans, in which the hydrophobic ends of the fatty acid tended to form together forming a micelle and the hydrophilic ends of the fructose protected the micelle formed from external influences by repulsive forces (Kokubun et al., 2013). These results suggest that acylated fructans could be used as foaming and stabilizing agents because these particles adsorb to the air-water interfaces (Dickinson, 2012).

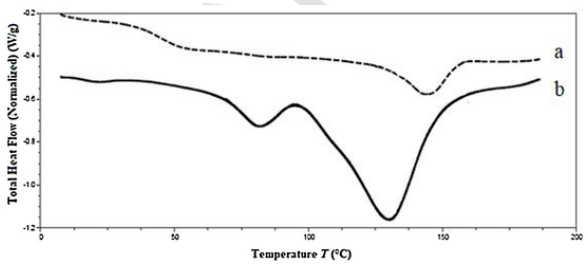

Fig. 3. Thermograms of native agave fructans (a) and acylated agave fructans (b) obtained by Differential Scanning Calorimetry with 5 K / min heating rate.

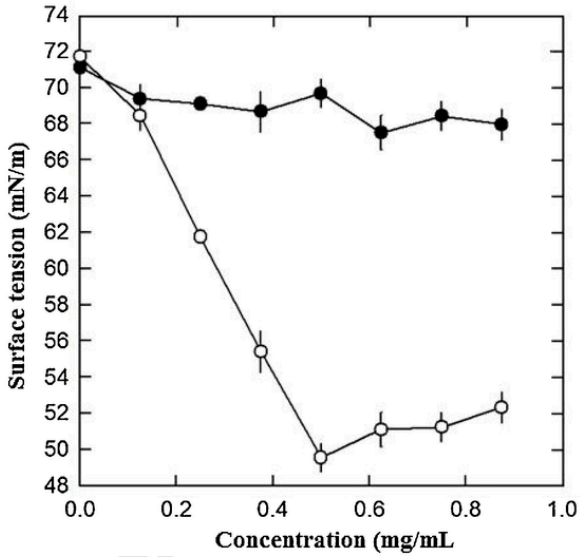

Fig. 4. Surface tension of native (- ● -) and acylated agave fructans (- ○ -) at different concentrations.

3.2. Physicochemical properties of native and modified fructans

Table 1 shows the effect of the modification on some physicochemical properties of modified fructans. The moisture content and water activity were 7 % and 0.4 in both samples. Such values are suitable to inhibit and microbial growth and to ensure a reduction in biochemical reactions (Bashir & Haripriya, 2016). The CIE L *, a *, b * color parameters of native and modified fructans were evaluated. Native and acylated fructans exhibited similar values in luminosity. However, acylation modified the values of parameters a* and b*. Acylated fruc-

Table 1
Physicochemical properties of native and modified agave fructans by acylation.

Properties	Native Agave Fructans	Modified Agave Fructans
Moisture content (g H_20/ 100 g)	5.12 ± 0.78 [a]	6.19 ± 0.58 [a]
Water activity (25 °C)	0.377 ± 0.01 [a]	0.377 ± 0.01 [a]
Color parameters		
L*	94.85 ± 0.21 [a]	94.02 ± 0.21 [a]
a*	−0.91 ± 0.21 [a]	0.03 ± 0.21 [b]
b*	6.14 ± 0.12 [a]	3.86 ± 0.12 [b]
Hue	81.50 ± 0.19 [a]	89.50 ± 0.19 [b]
Chroma	6.21 ± 0.12 [a]	3.86 ± 0.12 [b]
Total color change (ΔE)	–	2.54 ± 0.16
Water holding capacity (g water/g sample)	0.01 ± 0.04 [a]	3.40 ± 0.04 [b]
Oil holding capacity (g oil/g sample)	1.48 ± 0.01 [a]	5.18 ± 0.01 [b]

Values are mean of three replicates. Different letters within the same row indicate significant differences ($p < 0.05$) between samples.

tans had a lower value in parameter a* and a higher value in b*. These variations in the color parameters could be due to the incorporation of fatty acid in the fructans structure by the acylation reaction. The modification by incorporation of fatty acid did not have significant effect ($p > 0.5$) in the total color change, which is convenient for the possible addition in food. Acylated fructans showed an obvious reduction in water solubility. This reduction in solubility could be due mainly to a reduction in the negative total charges and to an increase in the hydrophobic interactions related to the fatty acid incorporation, which caused a reduction in the solvation degree and in hydrogen bonding in the aqueous medium (Mendoza-Sánchez et al., 2019).

Water and oil absorption capacity is the ability to absorb water or oil and is an important component with regard to beverage applications, bakery and other products, since it indicates the amount of water available in the gelatinization processes (Rengadu, Gerrano, & Mellem, 2020). In this work, the water and oil retention capacity are directly related to the intermolecular composition and organization of the polysaccharides that form the fructans. The minimum water retention capacity of native fructans is mainly due to its high-water solubility, which dissolve in its entirety. The water and oil retention capacity were significantly higher for acylated fructans ($p < 0.05$), since these showed a low water solubility and the formation of an agglomerate favored the entrapment of water and oil within its structure. It has been reported that oil absorption capacity involves physical entrapment of oil by capillary action that is favored by the porosity of the material (Kinsella & Melachouris, 1976). The improvement of the water holding capacity could be due to the fact that the incorporation of the lauric acid chains in the structure of the fructans produces a distribution of hydrogen bonds, steric hindrance and inter- and intra-particle disorganization favoring that the access of water to the amorphous region (Yadav & Patki, 2015), while the increase in oil retention capacity, could be due, in addition to these factors, to a greater chemical interaction between the oil and the hydrocarbon chains of the incorporated lauric acid.

3.3. Functional properties of native and modified fructans at different pH

Table 2 shows the solubility, emulsifying, swelling and foaming capacity of native and acylated agave fructans evaluated at three different pHs. Acylated fructans had a solubility index that ranged from 27.60 to 2.50 % at the different study pHs. The solubility index revealed that the acylation of fructans produced a drastic decrease in water solubility compared to native fructans, which is consistent with the decrease in reported solubility for starches acylated with lauric acid (Winkler, Vorwerg, & Wetzel, 2013). This is possibly due to the fact that the esterified lauric acid favors a greater rigidity of the structure of the fructans that affects its flexibility and degree of interaction with the hydroxyl groups of the water molecules, which is reflected in a decrease in the solubility of the modified fructans (Azfaralariff, Fazial, Sontanosamy, Nazar, & Lazin, 2020). The pH modifies the solubility of fructans, possibly because when the hydroxyl groups of the fructans are exposed to a solvent with a certain pH, the interactions between the hydroxyl groups are modified to a different degree, affecting their structure and physical properties. In turn, it has been reported that low pH tends to hydrolyze the glycosidic bonds producing smaller molecules or producing a rearrangement in their structure, while basic pH favors the breakdown of hydrogen bonds between polysaccharide chains. This, in turn, produces changes in its mobility and its structure that result in a greater possibility of association between the molecules and in a greater stability at a certain pH (Lee, Han, & Lim, 2009), which helps explain the variations in the functional properties of fructans at different pH values.

Table 2
Functional properties of native and acylated agave fructans evaluated at pH 4, 7 and 10.

Property	pH	Native Agave Fructans	Modified Agave Fructans
Solubility index (%)	4	100 [b]	15.45 ± 2.75 [a]
	7	100 [b]	27.60 ± 3.25 [a]
	10	100 [b]	2.65 ± 1.20 [a]
Emulsifying capacity (%)	4	–	16.22 ± 0.76
	7	–	8.22 ± 0.19
	10	–	–
Swelling capacity (mL/g)	4	0.01 ± 0.01 [a]	1.59 ± 0.08 [b]
	7	0.02 ± 0.01 [a]	1.54 ± 0.05 [b]
	10	0.03 ± 0.01 [a]	1.51 ± 0.03 [b]
Foaming capacity (%)	4	1.27 ± 0.09 [a]	25.33 ± 5.90 [b]
	7	1.16 ± 0.25 [a]	60.78 ± 4.23 [b]
	10	0.66 ± 0.18 [a]	40.90 ± 4.88 [b]
Foaming stability (min)	4	–	3.50 ± 0.35
	7	–	65.39 ± 0.50
	10	–	99.78 ± 0.21

Values are mean of three replicates. Different letters within the same row indicate significant differences ($p < 0.05$) between samples.
"—" no detectable.

Acylated fructans had an emulsifying capacity that varied from 8.22 to 16.22 % at pH of 4 and 7, respectively. These values were significantly higher ($p < 0.05$) compared to native fructans. This is possibly due to the fact that at these pHs there is an adequate hydrophilic-lipophilic balance between the hydroxyl groups of fructans and lauric acid, favoring the covalent bonds that increase the stability of the emulsion (Haque & Kito, 1983; Haque, Matoba, & Kito, 1982). Similarly, the swelling capacity of acylated fructans was greater than that exhibited by native fructans, suggesting that acylation produces changes in the structure by the incorporation of fatty acid, which results in a greater capacity to increase its volume by water adsorption (Raghavendra et al., 2004). The increase in swelling power might be attributed to the improved water percolation to the particles, afforded by the steric hindrance and repulsion of the introduced lauric acid hydrocarbon chain in the fructan structure (Shogren, Viswanathan, Felker, & Gross, 2000), evidencing a modification in its structure with respect to fructans unmodified. Possibly, the introduction of lauric acid in the fructan molecule increase swelling power because it can weaken the intermolecular association forces, favoring the entrapment of water in the cavities of the fructans particles increasing its volumen. The native fructans did not show foaming capacity. However, the foaming capacity of acylated fructans was significantly modified ($p < 0.05$) by the incorporation of fatty acid, reaching values of 25.33, 60.78 and 40.90 % by pH, 4, 7 y 10, respectively, indicating a high capacity to trap large volumes of air. The high foaming capacity was possibly due to the presence of lauric acid favoring intramolecular interactions which reduces the surface tension of fructans (Mendoza-Sánchez et al., 2019), or because the incorporation of fatty acid produces a partial split of the acylated macromolecule, which improves its amphiphilic properties and favors foam formation (Zmudzinski et al., 2014). The stability of the foam prevents its separation of phases and favors that the foam maintains its properties for a longer time. Acylated fructans exhibited a stability of 65.39 and 99.78 min for pH 7 and 10, which are similar to those reported for albumin (Naji-Tabasi & Razavi, 2016), suggesting that the use of this product could be used as a substitute for this protein in some food products. These changes in the properties of fructans at different studio pHs confirm that when a carbohydrate is dissolved in an aqueous medium, the pH affects its structure resulting in a change in its physical properties.

Fig. 5 shows the zeta potential of native and acylated agave fructans. It is observed that at low pH (pH < 1), both samples showed zeta potential values close to zero, favoring a greater agglomeration between the particles (Mendoza-Sánchez et al., 2019), which would help explain the protective behavior of fructans as prebiotics in

UNCORRECTED PROOF

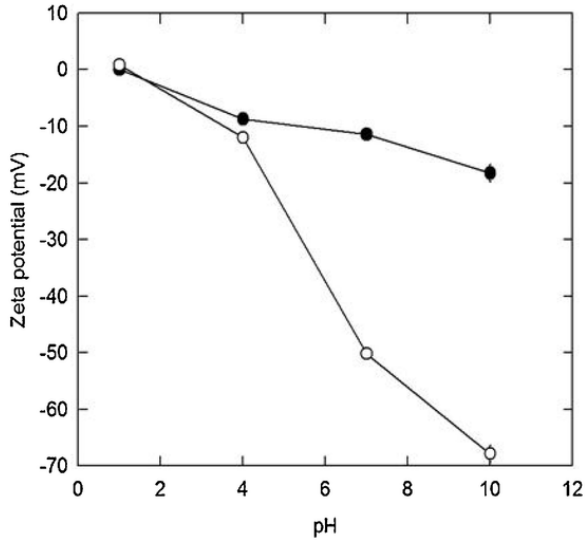

Fig. 5. Zeta potential of native agave fructans (- ● -) and acylated agave fructans (- ○ -) at different pH values.

pH stomach However, as the pH increased to pH 10, the zeta potential for native and acylated fructans decreased to −10 and −70 mV, respectively. The greatest decrease in zeta potential in modified fructans may be related to the presence of carboxylic acids present by the incorporation of lauric acid in its structure (Kokubun, Ratcliffe, & Williams, 2018), also demonstrating that at pH > 5 the zeta potential (<−30 mV), presents stable electrostatic interactions to the agglomeration or precipitation phenomena, favoring the stability of the solution (Acevedo-Guevara, Nieto-Suaza, Sanchez, Pinzon, & Villa, 2018; Mendoza-Sánchez et al., 2019).

4. Conclusions

This work demonstrated that native agave fructans can be successfully modified by acylation reacting with lauric acid in an aqueous solution, obtaining a high degree of substitution. The incorporation of the lauric acid molecule into the structure of native agave fructans was confirmed by FTIR and ^{1}H NMR. The resulting of hydrophobic acylated compound showed significant differences in the physicochemical properties, in the functional properties at the different studio pHs and changes in the thermal properties. The incorporation of lauric acid in the structure of fructans increased its emulsifying, foaming capacity and water and oil holding capacity and caused a decrease in the surface tension, presenting a critical concentration of aggregation exhibiting amphiphilic properties and changes in thermal properties. In general, acylated fructans have potential application in the use as an additive of a wide range of products such as ice cream, yogurt and various other foods and pharmaceutical products.

Acknowledgements

The authors acknowledge the support to CONACYT-Mexico for student grant number 860473 and Mieles Campos Azules, S. A. Company from Amatitán Jalisco for providing Native agave fructans.

References

Acevedo-Guevara, L, Nieto-Suaza, L, Sanchez, L T, Pinzon, M I, & Villa, C C (2018). Development of native and modified banana starch nanoparticles as vehicles for curcumin. International Journal of Biological Macromolecules, 111, 498–504. doi:10.1016/j.ijbiomac.2018.01.063.

Acosta-Domínguez, L, Hernández-Sánchez, H, Gutiérrez-López, G F, Alamilla-Beltrán, L, & Azuara, E (2016). Modification of the soy protein isolate surface at nanometric scale and its effect on physicochemical properties. Journal of Food Engineering, 168, 105–112. doi:10.1016/j.jfoodeng.2015.07.031.

Akin, M B, Akin, M S, & Kirmaci, Z (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream. Food Chemistry, 104, 93–99. doi:10.1016/j.foodchem.2006.11.030.

AOAC (1990). Official methods of analysis of the association of official analytical chemists (15th edition). Washington, DC.

Apolinário, A C, Martins de Carvalho, E, Goulart de Lima, B P, Dantas, P C, Converti, A, & Pessoa, A, et al. (2017). Extraction, isolation and characterization of inulin from *Agave sisalana* boles. Industrial Crops and Products, 108, 355–362. doi:10.1016/j.indcrop.2017.06.045.

Azfaralariff, A, Fazial, F F, Sontanosamy, R S, Nazar, M F, & Lazin, A M (2020). Food-grade particle stabilized pickerin emulsion using modified sago (*Metroxylon sagu*) starch nanocrystal. Journal of Food Engineering, 280, 109974. doi:10.1016/j.jfoodeng.2020.109974.

Bashir, M, & Haripriya, S (2016). Physicochemical and structural evaluation of alkali extracted chickpea starch as affected by γ-irradiation. International Journal of Biological Macromolecules, 89, 279–286. doi:10.1016/j.ijbiomac.2016.04.080.

Casas-Godoy, L, Arrizon, J, Arrieta-Baez, D, Plou, F J, & Sandoval, G (2016). Synthesis and emulsifying properties of carbohydrate fatty acid esters produced from *Agave tequilana* fructans by enzymatic acylation. Food Chemistry, 204, 437–443. doi:10.1016/j.foodchem.2016.02.153.

Cerdan-Leal, M A, Lopez-Alarcon, Ortiz-Basurto, R I, Luna-Solano, G, & Jimenez-Fernandez, M (2020). Influence of heat denaturation and freezing–lyophilization on physicochemical and functional properties of quinoa protein isolate. Cereal Chemistry, 97(2), 373–381. doi:10.1002/cche.10253.

Cruz-Cardenas, C I, Miranda-Ham, M L, Castro-Concha, L A, Ku-Cauich, J R, Verdauwen, R, & Reijinders, T, et al. (2015). Fructans and other water soluble carbohydrates in vegetative organs and fruits of different *Musa* spp. accessions. Frontiers in Plant Science, 6, 395. doi:10.3389/fpls.2015.00395.

Dickinson, E (2012). Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. Food Science and Technology, 24, 4–12. doi:10.1016/j.tifs.2011.09.006.

Espinoza-Andrews, H, & Urías-Silvas, J E (2012). Thermal properties of agave fructans (*Agave tequilana* Weber var. Azul). Carbohydrate Polymers, 87, 2671–2676. doi:10.1016/j.carbpol.2011.11.053.

FAC (Food additives and contaminants committee report on modified starches) (1980). Report 31. Ministry of agriculture. London, UK: Fishries and Food.

Gómez-Ordóñez, E, Jiménez-Escrig, A, & Rupérez, P (2010). Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. Food Research International, 43, 2289–2294. doi:10.1016/j.foodres.2010.08.005.

Grube, M, Beckers, M, Upite, D, & Kaminska, E (2002). Infrared spectra of some fructans. Spectroscopy, 16, 289–296. doi:10.1155/2002/637587.

Han, L, Ratcliffe, I, & Williams, P A (2017). Synthesis, characterisation and physicochemical properties of hydrophobically modified inulin using long-chain fatty acyl chlorides. Carbohydrate Polymers, 178, 141–146. doi:10.1016/j.carbpol.2017.09.008.

Haque, Z, & Kito, M (1983). Lipophilization of αs1-casein. 2. Conformational and functional effects. Journal of Agricultural and Food Chemistry, 31, 1231–1237. doi:10.1021/jf00120a022.

Haque, Z, Matoba, T, & Kito, M (1982). Incorporation of fatty acid into food protein: Palmitoyl soybean glycinin. Journal of Agricultural and Food Chemistry, 30, 481–486. doi:10.1021/jf00111a018.

Horton, J D, Goldstein, M S, & Brown, M S (2002). SREBPs: Activators of the complete program of cholesterol and fatty synthesis in the liver. The Journal of Clinical Investigation, 109(9), 1125–1131. doi:10.1172/JCI15593.

Kalepu, S, & Nekkanti, V (2015). Insoluble drug delivery strategies: Review of recent advances and business prospects. Acta Pharmaceutica Sinica B, 5(5), 442–453. doi:10.1016/j.apsb.2015.07.003.

Kawai, K, Fukami, K, Thanatuksorn, P, Viriyarattansak, C, & Kajiwara, K (2011). Effects of moisture content, molecular weight, and crystallinity on the glass transition temperature of inulin. Carbohydrate Polymers, 83, 934–939. doi:10.1016/j.carbpol.2010.09.001.

Kinsella, J E, & Melachouris, N (1976). Functional properties of proteins in foods: A survey. Critical Reviews in Food Science and Nutrition, 7(3), 219–280. doi:10.1080/10408397609527208.

Kokubun, S, Ratcliffe, I, & Williams, P A (2013). Synthesis, characterization and self assembly of biosurfactants based on hydrophobically modified inulins. Biomacromolecules, 14(8), 2830–2836. doi:10.1021/bm4006529.

Kokubun, S, Ratcliffe, I, & Williams, P A (2018). The interfacial, emulsification and encapsulation properties of hydrophobically modified inulin. Carbohydrate Polymers, 194, 18–23. doi:10.1016/j.carbpol.2018.04.018.

Lee, J H, Han, J A, & Lim, S T (2009). Effect of pH on aqueous structure of maize starches analyzed by HPSEC-MALLS-RI system. Food Hydrocolloids, 23, 1935–1939. doi:10.1016/j.foodhyd.2008.12.007.

Mancilla-Margalli, N A, & López, M G (2006). Water-soluble carbohydrates and fructan structure patterns from *Agave* and *Dasylirion* species. Journal of Agricultural and Food Chemistry, 54(20), 7832–7839. doi:10.1021/jf060354v.

Márquez-Aguirre, A L, Camacho-Ruiz, R M, Arriaga-Alba, M, Padilla-Camberos, E, Kirchmayr, M R, & Blasco, J L, et al. (2013). Effects of *Agave tequilana* fructans with different degree of polymerization profiles on the body weight, blood lipids and count of fecal *Lactobacilli/Bifidobacteria* in obese mice. Food & Function, 4(8), 1237–1244. doi:10.1039/c3fo60083a.

Mendoza-Sánchez, L G, Jiménez-Fernández, M, Melgar-Lalanne, M G, Gutiérrez-López, G F, Reyes-Espinosa, F, & Hernández-Arana, A, et al. (2018). Effect of enzymatic lipophilization on the functional properties of bovine alpha lactalbumin. Revista Mexicana de Ingeniería Química, 17, 331–347. doi:10.24275/uam/izt/dcbi/revmexingquim/2018v17n1/Mendoza.

Mendoza-Sánchez, L G, Jiménez-Fernández, M, Melgar-Lalanne, M G, Gutierrez-López, G F, Fernández-Arana, A, & Reyes-Espinoza, F, et al. (2019). Chemical

UNCORRECTED PROOF

lipophilization of bovine α-lactalbumin with saturated fatty acyl residues: effect on structure and functional properties. Journal of Agricultural and Food Chemistry, 67(11), 3256–3265. doi:10.1021/acs.jafc.8b05174.

Miramontes-Corona, C, Escalante, A, Delgado, E, Corona-Gonzalez, R I, Vazquez-Torres, H, & Toriz, G (2020). Hydrophobic agave fructans for sustained drug delivery to the human colon. Reactive & Functional Polymers, 146, 104396. doi:10.1016/j.reactfunctpolym.2019.104396.

Miramontes-Corona, C, Escalante, M A, Delgado, E, Corona-González, R I, Vázquez-Torres, H, & Toriz, G (2019). Characterization of modified agave fructans used as drug carriers to the colon by spectroscopy techniques. Proc. SPIE 11128, Infrared Remote Sensing and Instrumentation XXVII, 111280X. doi:10.1117/12.2526135.

Moreno-Vilet, L, Garcia-Hernandez, M, Delgado-Portales, R, Corral-Fernandez, N, Cortez-Espinosa, N, & Ruiz-Cabrera, M, et al. (2014). *In vitro* assessment of agave fructans from *Agave salmiana* as prebiotics and immune system activators. International Journal of Biological Macromolecules, 63, 181–187. doi:10.1016/j.ijbiomac.2013.10.039.

Morros, J, Levecke, B, & Infante, M R (2010). Chemical hydrophobic modification of inulin in aqueous media: Synthesis of -hydroxyalkyl ethers of inulin. Carbohydrate Polymers, 81, 681–686. doi:10.1016/j.carbpol.2010.03.039.

Naji-Tabasi, S, & Razavi, S (2016). New studies on basil (*Ocimum bacilicum* L.) seed gum: Part II—Emulsifying and foaming characterization. Carbohydrate Polymers, 149, 140–150. doi:10.1016/j.carbpol.2016.04.088.

Namazi, H, Fathi, F, & Dadkhah, A (2011). Hydrophobically modified starch using long –chain fatty acids for preparation of nanosized starch particles. Scientia Iranica, 18, 439–445. doi:10.1016/j.scient.2011.05.006.

Okunlola, A, Sarafadeen, A, & Adeyeye, M J (2017). Development of repaglinide microspheres using novel acetylated starches of bitter and Chinese yams as polymers. International Journal of Biological Macromolecules, 94, 544–553. doi:10.1016/j.ijbiomac.2016.10.032.

Ortiz- Basurto, R I, Rubio Ibarra, M E, Ragazzo Sanchez, J A, Beristain, C I, & Jiménez Fernández, M (2017). Microencapsulation of *Eugenia uniflora*L. juice by spray drying using fructans with different degrees of polymerisation. Carbohydrate Polymers, 175, 603–609. doi:10.1016/j.carbpol.2017.08.030.

Pintor-Jardines, A, Arjona-Román, J L, Totosaus-Sanchez, A, Severiano-Pérez, P, González-González, L R, & Escalona-Buendia, H (2018). The influence of agave fructans on thermal properties of low-fat, and low-fat and sugar ice cream. LWT. Food Science and Technology, 93, 679–685. doi:10.1016/j.lwt.2018.03.060.

Raghavendra, S N, Rastogi, N K, Raghavarao, K S M S, & Tharanathan, R N (2004). Dietary fiber from coconut residue: Effects of different treatments and particle size on the hydration properties. European Food Research and Technology, 218, 563–567. doi:10.1007/s00217-004-0889-2.

Rengadu, D, Gerrano, A S, & Mellem, J J (2020). Physicochemical and structural characterization of resistant starch isolated from *Vigna unguiculata*. International Journal of Biological Macromolecules, 147, 268–275. doi:10.1016/j.ijbiomac.2020.01.043.

Rogge, T M, Stevens, C V, Colpaert, A, Levecke, B, & Booten, K (2007). Use of acyl phosphonates for the synthesis of inulin esters and their use as emulsion stabilizing agents. Biomacromolecules, 485–489. doi:10.1021/bm060592z.

Santiago-García, P A, Mellado-Mojica, E, León-Martínez, F M, & Lopez, M G (2017). Evaluation of *Agave angustifolia* fructans as fat replacer in the cookies manufacture. LWT-Food Science and Technology, 77, 100–109. doi:10.1016/j.lwt.2016.11.028.

Shogren, R L, Viswanathan, A, Felker, F, & Gross, R A (2000). Distribution of octenyl succinate groups in octenyl succinic anhydride modified waxy maize starch. Starch-Stärke, 52(6–7), 196–204. doi:10.1002/1521-379X(200007)52:6/7<196::AID-STAR196>3.0.CO;2-4.

Singh, J, Kaur, L, & McCarthy, O J (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemicalle modified starches for food applications – a review. Food Hydrocolloids, 21, 1–22. doi:10.1016/j.foodhyd.2006.02.006.

Toriz, G, Delgado, E, & Zuñiga, V (2007). A proposed chemical structure for fructans from blue agave plant (*tequilana Weber* var. azul). e-Gnosis, 5, 1.

Urías-Silvas, J E, Cani, P D, Delmée, E, Neyrinck, A, López, M G, & Delzenne, N M: (2008). Physiological effects of dietary fructans extracted from *Agave tequilana* Gto. and Dasylirion spp. The British Journal of Nutrition, 99, 254–261. doi:10.1017/S0007114507795338.

Wani, I, Sogi, D, Wani, A, & Gill, B (2013). Physico-chemical and functional properties of flours from Indian kidney bean (*Phaseolus vulgaris L.*) cultivars. LWT-Food Science and Technology, 53, 278–284. doi:10.1016/j.lwt.2013.02.006.

Winkler, H, Vorwerg, W, & Wetzel, H (2013). Synthesis and properties of fatty acid starch ester. Carbohydrate Polymers, 98, 208–216. doi:10.1016/j.carbpol.2013.05.086.

Yadav, D K, & Patki, P E (2015). Effect of acetyl esterification on physicochemical properties of chick pea (*Cicer arietinum* L.) starch. Journal of Food Science and Technology, 52(7), 4176–4185. doi:10.1007/s13197-014-1388-5.

Yang, L, Zhou, Y, Wu, Y, Meng, X, Jiang, Y, & Zhang, H, et al. (2016). Preparation and physicochemical properties of three types of modified glutinous rice starches. Carbohydrate Polymers, 137, 305–313. doi:10.1016/j.carbpol.2015.10.065.

Zmudzinski, D, Ptaszek, P, Kruk, J, Kaczmarczyk, K, Roznowski, W, & Berski, W, et al. (2014). The role of hydrocolloids in mechanical properties of fresh foams based on egg white proteins. Journal of Food Engineering, 121, 128–134. doi:10.1016/j.jfoodeng.2013.08.020.

Zotarelli, M F, Martins-Da Silva, V, Durigon, A, Dupas-Hubinger, M, & Borges-Laurindo, J (2017). Production of mango powder by spray drying and cast-tape drying. Powder Technology, 305, 447–454. doi:10.1016/j.powtec.2016.10.027.

UNCORRECTED PROOF