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Evaluación de la fermentación de fructanos de agave (agavinas) acetilados, con *Saccharomyces boulardii* como probiótico

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Abstract

Agave fructans (agavins) are fructose polymers that possess β (2-1) and β (2-6) bonds. Thus, they cannot be hydrolyzed by digestive enzymes and are hence classified as indigestible oligosaccharides. These molecules have gained importance due to their various applications, one of which is their prebiotic capacity. They have also been used as wall material, and encapsulating bioactive compounds. The paper aim to characterize and modify the native fructanos (NF) of Agave angustifolia Haw by acetylation and evaluate their fermentation with *Saccharomyces boulardii* yeast.To determine the degree of polymerization of the native fructans, FTIR, ^{1}H NMR, and MALDI-TOF-MS were used for characterization. They were subsequently modified and tested as a carbon source with *S. boulardii*, which was used as a probiotic. The fructans used had a degree of polymerisation (DP) ranging from 4-10 units. Acetylation was performed with a change in functional groups (C = O) in the 1700-1750 cm^{-1} region, indicating that reaction was successful. Thermal properties changed due to relaxation of the amorphous structure of fructanos during acetylation. Furthermore, yeast cell development with modified agave fructans (7.32 $\log_{10}$ CFU/mL and native agave fructans (7.09 $\log_{10}$ CFU/mL) indicated that the compound's fermentation was unaffected.
Keywords: Agave angustifolia Haw., fructans, acetylation, fermentation.

Resumen

Los fructanos de agave (agavinas) son polímeros de fructosa que poseen enlaces β (2-1) y β (2-6). Esta característica no permite su hidrólisis por enzimas digestivas y los clasifica como oligosacáridos indigeribles. Estas moléculas han cobrado relevancia por sus diferentes usos, uno de los cuales es su capacidad prebiótica, también se han utilizado como material de pared, formando encapsulados con componentes bioactivos. Este trabajo tiene como objetivo caracterizar y modificar los fructanos nativos (NF) de Agave angustifolia Haw por acetilación y evaluar su fermentación utilizando la levadura *Saccharomyces boulardii*. Los fructanos nativos se caracterizaron mediante FTIR, ^{1}H NMR y MALDI TOT-MS, para determinar su grado de polimerización. Posteriormente, se evaluaron utilizándolos como fuente de carbono por *S. boulardii* utilizado como probiótico. Los fructanos utilizados tuvieron un DP de 4-10 unidades, también se encontró un cambio en los grupos funcionales (C = O) en la región de 1700-1750 cm^{-1}, lo que indica que la reacción fue exitosa. Las propiedades térmicas cambiaron debido a la relajación de la estructura amorfa de los fructanos durante la acetilación. Además, el desarrollo de células de levadura con fructanos de agave modificados (7,32 Log_{10} UFC / mL) y fructanos de agave nativos (7,09 $\log_{10}$ UFC / mL) indica que la fermentación del compuesto no se vio afectada.
Palabras clave: Agave angustifolia Haw, fructanos, acetilación, fermentación.

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1 Introduction

Different authors have reported prebiotic activity of agave fructans in more than one Agave plant species such as *A. tequilana*, *A. salmiana*, *A. atrovirens* and *A. angustifolia* (Moreno-Vilet *et al*., 2014; Mueller *et al*., 2016; Ceja-Medina *et al*., 2021.) and although this prebiotic activity has been validated with the fermentability of various probiotic microorganisms including bacteria and yeasts. There are still a limited number of reports on the probiotics yeastes using agave fructans as prebiotic. Agavins have primarily been studied for their prebiotic capacity, because not digested by enzymes found in the gastric tract (García-Gamboa *et al*., 2020), due to they stimulate the growth of bacteria in the colon and the production of short-chain fatty acids, benefiting gastrointestinal health. (Velázquez-Martínez *et al*., 2014; Andrade *et al*., 2018; Alvarado-Jasso, *et al*., 2020).

Fructans are fructose polymers derived from the sucrose molecule, which is a fructose-glucose disaccharide. The type of fructan is determined from the nature of linkage between adjacent fructose units, the position of glucose residues, and the degree of polymerization, which can range from three to hundreds of fructose units, with a wide range of linkages and fructosyl residues (Franco-Robles & López, 2015; Rodríguez-González *et al*., 2019). Fructans-type agavins extracted from agave plants show branching characterized by two different linkages between fructose monomers, β (2-1) and β (2-6), and may internally contain glucose (Nava-Cruz *et al*., 2015; Santiago-García *et al*., 2021). Because fructans have higher water absorption (Ortiz-Basurto *et al*., 2017; Ignot-Gutiérrez *et al*., 2020), these bonds confer properties such as high solubility, hygroscopicity, and stickiness due to the hydrogen bonding of water with the hydroxyl groups of carbohydrates (Romano *et al*., 2018).

These properties limit the technological applications of such molecules in the chemical and pharmaceutical industries (Ignot-Gutiérrez *et al*., 2020). Furthermore, due to their characteristic branching, this molecule was investigated for its ability to encapsulate agavins. Delgadillo *et al*. (2015), made microspheres by coacervation of native *Agave tequilana* fructans and their esterification to change the solubility and encapsulate a drug (ibuprofen), finding promising results for using these fructans in drug delivery.

As a result, it is necessary to modify their functional properties by changing some physicochemical properties, allowing them to be used in various applications such as the food industry, tissue engineering, and drug delivery and controlled release (Verma *et al*., 2021). The aim is to use free carbonyl and hydroxyl groups scattered along the native polysaccharides skeleton to make chemical modifications in order to obtain molecules with specific properties suited to non hydrophilic applications. In this regard, the use of chemical modifications in polysaccharides such as gums, pectins, starch and fructans, has been an alternative for used it wall material because they are chemically versatile due to their hydrophilic groups, which can generate non-covalent bonds and thus become more available. These polysaccharides also exhibit characteristics such as biodegradability (Mizrahy and Peer, 2012; Liu *et al*., 2015; Fertah *et al*., 2015; Luo *et al*., 2021). However, when these types of modifications are applied to polysaccharides with prebiotic capacity, their chemical structure can be affected. Therefore, this study aims to characterize the native fructans (NF) modified by acetylation extracted of Agave angustifolia Haw and evaluate their fermentation with *Saccharomyces boulardii* as probiotic yeast.

2 Materials and methods

2.1 *Obtaining and characterizing NF from Agave angustifolia Haw*

Six-year-old pineapples of *Agave angustifolia* Haw. were collected from Ejido La Mezquitera in the municipality of Jojutla, Morelos, México (18°33'43.0" N and 99°06'01.0" W). Agave fructans aqueous extract were obtaing using a modular pilot system (Patent MX/a/2015/016512) designed by a group of researchers from CeProBi. This system produced a liquid extract with a high fructans content, which was spray-dried using a Mobile MinorTM 2000 spray dryer (GEA Niro, Denmark) using a dual-fluid, parallel-flow pneumatic nozzle dryer with an inlet temperature of 200 °C, outlet temperature of 70 °C, and an atomization air pressure of 1 kg/cm^2. Total carbohydrates were calculated using the methodology described by Dubois *et al*. (1956), with a glucose-type curve for quantitative evaluation. Reducing sugars were quantified using the methodology proposed by Miller (1959) with fructose as a standard.

2.1.1 *Determination of reducing sugars by HPTLC*

The reducing sugars of agave fructans extract were analyze by HPTLC on a CAMAG instrument equipped with a CAMAG® Linomat 5 semi-automatic autosampler. The fructan sugars were identified using the methodology by Mancilla-Margalli & Lopez (2006). Fructans solutions were prepared. Glucose, fructose, and sucrose solutions (3 mg/mL) were used as standards. Subsequently, the plates were developed twice at 1 h intervals, with a mobile phase comprising a butanol:methanol:water ratio of 3:12:4 (v/v/v). After heating the plates at 180 °C for 20 min, the sugars were developed using a developer composed of diphenylamine/aniline/acetone/phosphoric acid (0.4:0.4:16:3 v/v/v/v). Finally, after 5 min of heating the plates at 120 °C, bands of different colors were observed.

2.1.2 MALDI-TOF analysis

MALDI-TOF was used to determine the average molecular weight of NF in a micrOTOF-Q mass spectrometer (Bruker Daltonics, USA) equipped with a positive reflector nitrogen laser. The samples were dissolved in water (1 mg/mL) and then incorporated into a 2,5-dihydroxybenzoic acid matrix.

2.2 Acetylation of agave fructans

According to Wu and Lee's (2000) methodology, NF were acetylated in a reaction using N,N-dimethylformamide (DMF) (Fermont) as a suspension medium by reacting the NF with acetic anhydride (AA) at 40 °C for up to 24 h. To obtain modified fructans (MF), 2 g of sample (NF)was taken, and AA was added, followed by sodium acetate (AcNa) as a catalyst (0.05% w/w). The reaction product was precipitated with an excess amount of water and centrifuged at 11700 rpm for 10 min at 10 ° C (HERMLE - Z 383 K, Germany) after which the MF was lyophilized (Lyophilizer, Labconco).

Fourier transform infrared spectroscopy (FTIR) was used to analyze NF and MF samples using a Shimadzu Spectrophotometer (IRAffinity model, Shimadzu, Japan), with an ATR accessory, in transmission mode. Transmission spectra in the spectral range 400-4500 cm^{-1} were recorded with a resolution of 4 cm^{-1}.

2.3 Optimization of chemical modification conditions

An experimental design was conducted with the following selected independent variables: DMF concentration (1, 5, 10, 15 mL), AcNa (1.2, 4.8, 3, 6.6 mL), AA (2.6, 6.5, 10.4, 14.3 mL), NF (0.5, 1.25, 2, 2.75 g) and MF as a dependent variable. The analysis was conducted using the MiniTab V.18 software with the response surface (annex) to determine which variables affect the acetylation reaction.

2.3.1 Nuclear Magnetic Resonance

The NF and MF ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in D2O using a spectrometer, model Avance 750 MHz (Bruker, Bremen, Germany), at 750 MHz for ^{1}H and 188.62 MHz for ^{13}C. The spectra were acquired after 64 scans with a relaxation delay of 1.0 s and a spectral width of 15000 Hz.

2.3.2 Thermal properties

Differential Sacanning Calorimetry (DSC) of NF and MF were measured in a nitrogen atmosphere using DSC equipment (TA Instruments, Q1000, New Castle, USA). Thermograms were recorded in the temperature range of 25 to 300 °C at a heating rate of 5 °C min^{-1}. All measurements were taken three times.

2.3.3 Modified fructanos fermentation with Saccharomyces boulardii

The following five substrates were tested: fructans native (NF), fructans modified (MF), glucose (GLU), fructose (FRU), (carbon source concentration of 10 g/L) and without carbon source (WCS); the last three were used as controls culture medium. The culture media were sterilized in an autoclave for 20 min at a temperature of 120 °C and a pressure of 15 psi. All treatments were made in triplicate. The probiotic yeast strain *Saccharomyces boulardii* used was obtained from Floratil® (Merk, S.A. de C.V., Mexico). To activate the strain, the lyophilized content of a capsule was poured into 50 mL of yeast-extract-peptone-dextrose (YPD) broth (0.5 g/L NaCl, 10 g/L glucose, casein peptone 5 g/L, yeast extract 5 g/L), and agar-agar (15 g/L) at pH ± 6.7 was used if the solid medium was required. The incubation temperature of 37 °C and the shaking rate of 200 rpm was kept constant throughout the 6 h process (Ávila-Reyes *et al*., 2016). Subsequently, an aliquot was taken to inoculate sterile 5% YPD broth and incubate for 12 h under the above conditions. Subsequently, the inoculum for fermentation was detected with a Shimadzu UV spectrophotometer (Model UV-1800, Japan) at 640 nm.

To characterize the behavior of yeast in YPD culture broth incubated at 37 °C and 200 rpm, a typical curve was performed. Cell biomass was measured using an optical density spectrophotometer at 640 nm. Cell viability was determined by measuring colony forming units per mL (CFU/mL) after diluting 100 μL of sample in sterile phosphate buffered saline (PBS) and making serial dilutions to seed on YPD agar using the microdroplet technique at 0, 1, 2, 3, 4, 10, 24, 36, and 50 h (Miles and Misra, 1938).

Using the methodology described in the preceding sections, the treatments were inoculated at 5% with the active yeast strain *Saccharomyces boulardii*. The growth conditions were set in a Lab-Line Orbit Incubator-Shaker incubator at 37 °C and 200 rpm. The OD was measured in a spectrophotometer at 640 nm after 0, 2, 4, 6, 24, 30, and 48 h of inoculation. Subsequently, live and dead cells were observed using a confocal laser scanning microscope (CLSM) model LSM 800 (Carl Zeiss, Germany). Viability staining was performed on the samples following Jones & Senft (1985), with some modifications; propidium iodide (YP) and carboxyfluorescein diacetate (cFDA) were used.

2.3.4 Statistical analysis

Statistical AnalysisResults were analyzed with SPSS Statistics version 20 software and expressed as mean ± standard error (SEM). Statistical significance was determined by analysis of unidirectionalvariance (ANOVA). Values having $p < 0.05$ were considered significant.

3 Results and discussion

3.1 Carbohydrates quantification

The carbohydrate content was determined spectrophotometrically; the NF had a reducing sugar content of 35.7 mg/g sample and total sugar content of 429.5 mg/g; statistical analysis revealed no significant difference between the samples. This content was similar to that reported by Mancilla-Margalli and Lopez, (2006). They reported total carbohydrate contents ranging from 360 to 640 mg/g of six different species (*A. tequilana* grown in Jalisco and Guanajuato and *Agave fourcroydes*) used in different parts of Mexico. According to those authors, this variation could be attributed to abiotic factors such as climate and soil. Our carbohydrate content results, on the other hand, suggest that these species are sources of water-soluble carbohydrates. The reducing sugar content obtaining by HPTLC technique was 25.1 mg/g sucrose, 0.045mg/g glucose, and 52.44 mg/g fructose. Since according to Montañez-Soto *et al*. (2011), the content of reducing sugars in fructan extracts is the result of the leaching conditions (temperature and time), the organs used (leaves, stem, etc.) and the age of the plant.

3.1.1 MALDI-TOF analysis

The intensity of the peaks around 527-10137 Da was revealed by analyzing the MALDI-TOF-MS spectrum. This indicates a degree of polymerization (DP) between 5 and 10 units. Further analysis revealed that these fructans are short polysaccharides with predominant oligosaccharides content. Finally, it was demonstrated that these fructans are an amorphous material with a complex structure, containing linear chains with β $(2 \rightarrow 1)$ bonds and branched chains with β $(2 \rightarrow 6)$ bonds. These DPs agree with those reported by Arrizon *et al*. (2010), who discovered in the spectra peaks with intensities ranging from 680 to 1826.3 Da, corresponding to DP ranging from 4 to 11 units.

3.2 Chemical modification of agave fructans by acetylation

The substitution of the hydroxyl groups of agave fructans with acetyl groups of AA allowed them to be identified via a comparison of the infrared spectra of NF (Figure 1) with inulin as a reference. The hydroxyl groups belonging to carbohydrates are found at 3292 cm^{-1}. C-H stretching is shown in the region 2922-2941 cm^{-1} (Velázquez-Martínez *et al*., 2014, Apolinário *et al*., 2017). The band around 1415 cm^{-1} could be attributed to the deformation of CH_2-OH in the fructose ring. C-O-C groups from glucose and glucosidic bonds from fructans were observed in the 800-1200 cm^{-1} region (Pintor-Jardines *et al*., 2018).

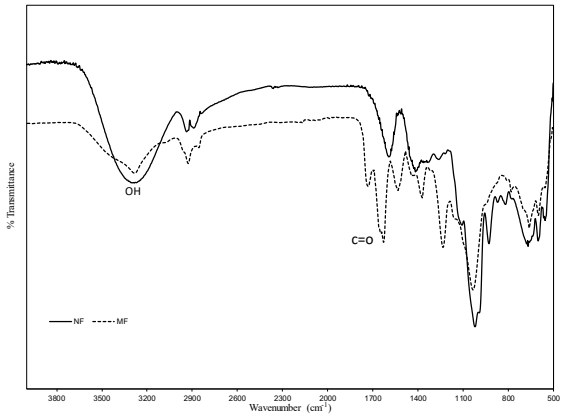

Figure 1. FTIR spectrum of native inulin, modified inulin, native fructans (NF), and modified fructans (MF) .

The MF show functional group changes in the 1700-1750 cm^{-1} region. These results indicated that both samples underwent acetylation. Walz *et al*. (2018) discovered a bending vibration of the CH_2-CH_3 bonds of inulin during acetylation in the region of 1220 cm^{-1}, and defined it as an important characteristic of this reaction. Furthermore, they discovered that in the region of 3500 cm^{-1}, the modified samples' band intensities decreased. This result is consistent with that reported by Miramontes-Corona *et al*. (2020), who studied acetylation in fructans from *A. tequilana* Weber and discovered that this modification aided in the encapsulation of a drug (ibuprofen).

3.2.1 Optimization of chemical modification conditions

According to the response surface graphs, the variation of DMF as dispersion medium and AA as donor molecule of acetyl groups at high concentrations negatively affected the reaction (Figure 2a). Aditionally the reaction medium (DMF) concentration was a determinant for possible fructan hydrolysis. Since when NF was exposed to high concentrations and prolonged times of DMF (20 ml), the acetylated fructans were hydrolyzed to reducing sugars leading to Maillard reactions with traces of NF proteins (Figure 2b) and this was possible to observe by browning of the supernatant in the reaction.

Therefore, AA and NF are the main precursors for the NF modification reaction (Figure 2c). Finally the optimal formulation was obtained through surface design analysis to obtain a 52% yield in the reaction according to the Eq.1, with a correlation adjustment r2=0.9699. Similary yield were reported by Walz *et al*. (2018), when they modified inulin via acetylation and obtained a yield between 11% and 56%, depending on the degree of substitution in that molecule.

$$MF = (0.168 + 0.1403DMF - 0.1686AA + 0.1843NF - 0.00567DMF) \times (DMF + 0.01043AA) \times AA \quad (1)$$

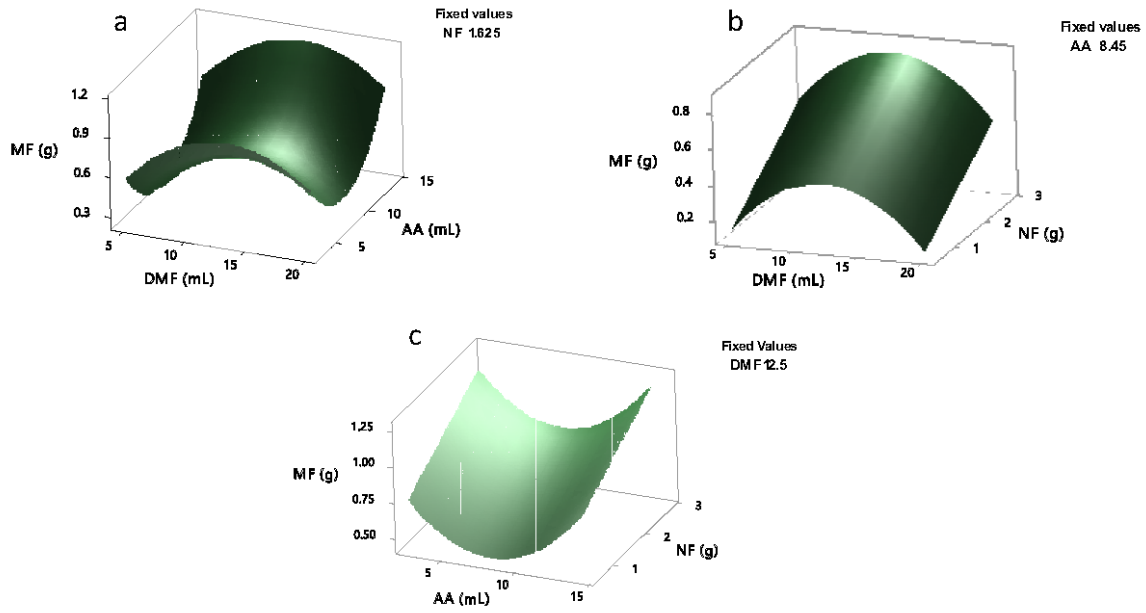

Figure 2. Surface response graphs for the modification reaction MF: a) MF vs NF, AA, b) MF vs DMF, NF c) MF vs. AA, DMF.

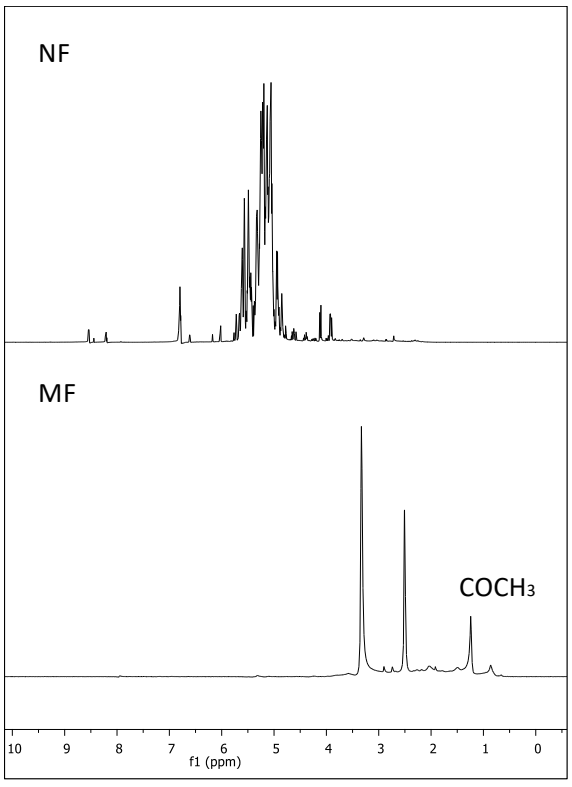

Figure 3. ^{1}H NMR spectra of NF (in D_2O) and MF (in DMSO).

3.2.2 Nuclear Magnetic Resonance

The ^{1}H spectra (Figure 3) shows that changes were found in a residue of α-D-Glcp type bonds, with the NF having a displacement of δ 5.23, which agrees with that reported by Lopez *et al*. (2003), who described the structure of *A. tequilana* in the 4.2 ppm region, where the anomeric carbon of a monosaccharide is present. According to Lopez *et al*. (2003), at 103.68 ppm in both agaves, C-2 units corresponding to β-bond bonds (2 $\rightarrow$ 1) were observed, as was C-6 in the 60-64 ppm region, which is characteristic of β bonds (2 $\rightarrow$ 6). Apolinário *et al*. (2017) describe an important region in Agave sisilana, from 75-80 ppm, where C-5 signals can be found. Finally, the 81.6 ppm signal can be attributed to the β bond (2 $\rightarrow$ 6). At 1.22 ppm, acetylated fructans show methyl group-related signals. This confirms that fructans had been modified.

3.2.3 Thermal properties

DSC was employed to analyze the thermal transitions of NF and MF (Figure 4). The glass transition temperature (Tg) of NF was 145 °C. Because of their two types of bonding, β (2-6) and β (2-1), agave fructans are thought to have a complex structure (Mancilla-Margalli, & Lopez, 2006). After modification MF Tg value decreased to 104 °C and according to Ignot-Gutierrez *et al*. (2020) and Walz *et al*. (2018), who observed lower Tg values in chemically modified inulin-type fructans and agavins, could be due to the presence of monosaccharides as products of NF hydrolysis by the use of DMF as reaction medium or by a possible decrease of molecular weight in the MFs during chemical modification.

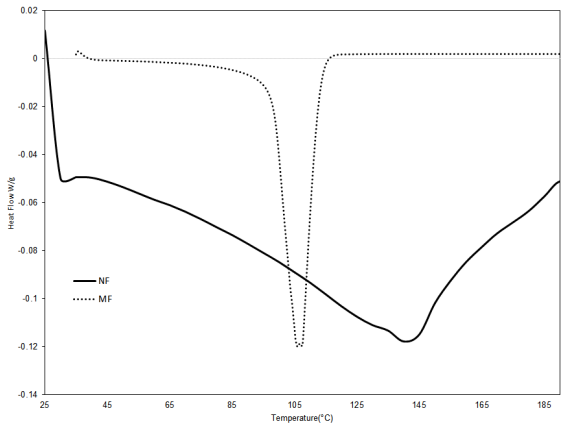

Figure 4. DSC thermograms of NF and acetylated fructans, MF.

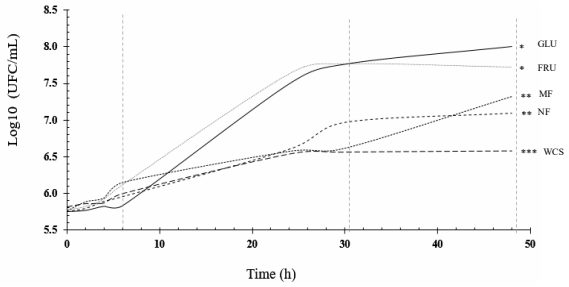

Figure 5. Cell growth of *S. boulardii* ($\log_{10}$ CFU/mL) in culture media supplemented with different carbon sources: without carbon source (WCS), glucose (GLU), fructose (FRU). Samples taken at 0, 2, 4, 6, 24, 30, and 48 h. ***SFC $p<0.05$ at 48 h. *GLU, FRU $p<0.05$. **MF, NF $p<0.05$. (*, ** and ***) indicate significant difference between culture media.

3.2.4 Prebiotic properties with Saccharomyces boulardii

The results obtained from the yeast kinetics in the presence of different carbon sources are presented in figure 5; At 48 h, the culture medium supplemented with GLU had a CFU increased of 8.01 $\log_{10}$ (CFU/mL), whereas that of FRU was 7.88 $\log_{10}$(CFU/mL). The culture media with NF 7.09 $\log_{10}$(CFU/mL) and MF 7.32 $\log_{10}$(CFU/mL) did not show significant differences ($p < 0.05$), but they were different with respect to cell growth in the media with GLU and FRU, which had the highest biomass. On the other hand, *S. boulardii* had the slowest growth in the culture medium without added carbohydrates (WCS), 6.58 $\log_{10}$(CFU/mL). All of the culture media tested were significant differences compared with the medium without a carbon source. According to Hatoum *et al*. (2012) and Mitterdorfer *et al*. (2001), yeasts prefer to ferment simple carbohydrates or disaccharides (glucose, fructose, mannose, maltose, or sucrose), which explains why less growth was obtained in the culture media containing NF and MF.

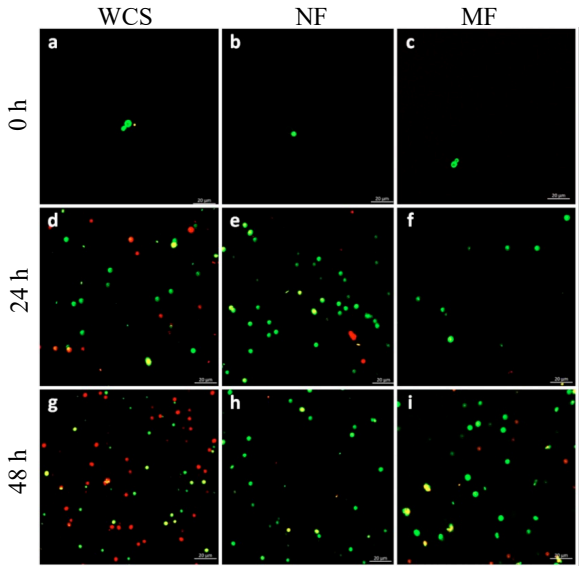

Figure 6. Images of *S. boulardii* by CLSM: WCS, NF, and MF. a) WCS 0 h, b) NF 0 h, c) MF 0 h, d) WCS 24 h, e) NF 24 h, f) MF 24 h, g) WCS 48 h, h) NF 48 h, and i) MF 48 h. 20-μm scale bar.

The medium formulated with MF was 0.23 $\log_{10}$(CFU/mL) faster growth than the medium formulated with NF. This faster growth maight be related with a less degree of polymerization (DP) of MF by hydrolysis; Blohm and Heinze 2019, report that carbohydrate DP is affected after chemical treatment, indicating depolymerization.

The NF fermentation exhibited a mildly pronounced exponential phase until about 30 h, when it transitioned to a stationary phase. Between 24 and 30 h after inoculation, *S. boulardii* showed a small stationary phase, but after this time and up to 48 h, it marked a pronounced exponential growth that places the biomass increase in this culture medium above the NF. On the other hand, Arrizon *et al*. (2014) report in their fermentation study of *S. boulardii* measured by D.O. at 490 nm for different carbon sources. The exponential phase begins between 2 and 8 h for the fermentation of fructans from *A. tequilana*, and 4 h for inulin. After 12 h, both fermentations reached their stationary phase. Similarly, Velázquez *et al*. (2014) and Gómez *et al*. (2010) mention that characteristics such as the degree of polymerization and the structure of the compound to be fermented influence probiotic microorganisms' behavior, and these characteristics are related to their ability to ferment carbon sources.

In the initial fermentation stage, the samples observed under the CLSM of the culture media: WCS, NF, and MF, show differences in cell viability, with bright green spots observed due to staining with carboxyfluorescein diacetate cFDA (Figure 6a-c). After 24 h, cell viability was compromised in the absence of carbohydrates, propidium iodide (YP) stains non-viable cells or those with membrane damage, fluorescing red (Figure 6d-f). Cell viability is

severely compromised in the absence of fermentable carbohydrates, as seen in WCS; where a large number of cells did not show viability, the result stained red, as reported by Ávila *et al*. (2016). The yellow coloration is caused by superimposing the laser that excites YP and cFDA. It indicates that the cells suffered membrane damage but retained metabolic activity, indicating that the cells were still dormant. For NF and MF, cell viability was found to be similar at 48 h (Figure 6g-i), with fluorescence thanks to cFDA staining and confirming the previous growth characteristics.

Conclusions

Chemical modification was observed in the infrared region of the spectral characteristics of carbonyl groups. It was also discovered that increasing the DMF content in the reaction causes hydrolysis in the molecule, which is unfavorable for modification. The optimization resulted in a maximum yield of 52%. Finally, when using yeast such as *Saccharomyces boulardii*, acetylation did not change the prebiotic activity of NF, because of the yeast is able to use MF as a carbon source, but it did change its thermal properties due to relaxation of the amorphous structure of fructans during acetylation.

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References

Alvarado-Jasso, G.M., Camacho-Díaz, B.H., Arenas-Ocampo, M.L., Jiménez-Ferrer, J.E., Mora-Escobedo, R. and Osorio-Díaz, P. (2020). Prebiotic effects of a mixture of agavins and green banana flour in a mouse model of obesity. *Journal of Functional Foods 64(May)*, 103685. https://doi.org/10.1016/j.jff.2019.103685

Apolinário, A.C., de Carvalho, E.M., de Lima Damasceno, B.P.G., da Silva, P.C.D., Converti, A., Pessoa, A. and da Silva, J.A. (2017). Extraction, isolation and characterization of inulin from *Agave sisalana* boles. *Industrial Crops & Products 108*, 355-362. https://doi.org/10.1016/j.indcrop.2017.06.045

Andrade, A.I.C., Bautista, C.R., Hernández, C.G., Cabrera, M.A.R., Ahumada, C.F., Chávez, E.G. and Lagunes, A.G. (2018). International Journal of Biological Macromolecules Physiometabolic effects of Agave salmiana fructans evaluated in Wistar rats. *International Journal of Biological Macromolecules 108*, 1300-1309. https://doi.org/10.1016/j.ijbiomac.2017.11.043

Arrizon, J., Hernández-Moedano, A., Toksoy-Oner, E. and González-Avila, M. (2014). In vitro prebiotic activity of fructans with different fructosyl linkage for symbiotics elaboration. *International Journal of Probiotics and Prebiotics 9(3)*, 69-76.

Arrizon, J., Morel, S., Gschaedler, A. and Monsan, P. (2010). Comparison of the water-soluble carbohydrate composition and fructan structures of *Agave tequilana* plants of different ages. *Food Chemistry 122*, 123-130. https://doi.org/10.1016/j.foodchem.2010.02.028

Ávila Reyes, S.V., Camacho-Díaz, B., Acosta García, M.C., Jiménez-Aparicio, A.R. and Hernández Sánchez, H. (2016). Effect of salt and sugar osmotic stress on the viability and morphology of *Saccharomyces boulardii. International Journal of Environment, Agriculture and Biotechnology 1(3)*, 593-602. http://dx.doi.org/10.22161/ijeab/1.3.43

Blohm, S. and Heinze, T. (2019). Studies about the acylation of starch in dipolar aprotic solvents. *Starch 71(9-10)*, 1-8. https://doi.org/10.1002/star.201900053

Ceja-Medina, L. I., Medina-Torres, L., Gonzáles-Ávila, M., Martínez-Rodriguez, J. C., Andrade-González, I., Calderón-Santoyo, M., Ragazzo-Sánchez, J. A., & Ortiz-Basurto, R. I. (2021). In vitro synbiotic activity of lactobacillus plantarum encapsulated with mixtures of Aloe vera mucilage, agave fructans and food additives as wall materials. *Revista Mexicana de Ingeniería Química, 20(2)*, 711-723. http://www.redalyc.org/articulo.oa?id=62029966013

Delgadillo, E., Corona, R.I., Toriz, G., Contreras, H.J., Sadeghifar, H., Baobing, W., Yang, G., Lucia, L.A. and Delgado, E. (2015). Coacervated liposoluble fructan-based host-guest microspheres as unique drug delivery materials. *RSC Advances 5 (83)*, 67759-67766. https://doi.org/10.1039/C5RA10164F

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry 28*, 350-356. https://doi.org/10.1021/ac60111a017

Espinosa-Andrews, H. and Urias-Silvas, J.E. (2012). Thermal properties of agave fructans (*Agave tequilana* Weber var. Azul). *Carbohydrate Polymers 87(4)*, 2671-2676. https://doi.org/10.1016/j.carbpol.2011.11.053

Fertah, M., Belfkira, A., Taourirte, M. and Brouillette, F. (2015). Controlled release of diclofenac by a new system based on a cellulosic substrate and

calcium alginate. *Bio Resources 10(1950)*, 5932-5948. https://doi.org/10.15376/biores.10.3.5932-5948

Franco-Robles, E. and López, M.G. (2015). Implication of fructans in health: Immunomodulatory and antioxidant mechanisms. *Scientific World Journal*. http://dx.doi.org/10.1155/2015/289267

García-Gamboa, R., Gradilla-Hernández, M. S., Ortiz-Basurto, R. I., García-Reyes, R. A., & González-Avila, M. (2020). Assessment of intermediate - and long- chains agave fructan fermentation on the growth of intestianl bacteria cultured in a gastrointestinal tract simulator. *Revista Mexicana de Ingeniería Química 19(2)*, 827-838. https://doi.org/https://doi.org/10.24275/rmiq/Bio842.

Gomez, E., Tuohy, K.M., Gibson, G.R., Klinder, A. and Costabile, A. (2010). In vitro evaluation of the fermentation properties and potential prebiotic activity of Agave fructans. *Journal of Applied Microbiology 108(6)*, 2114-2121. https://doi.org/10.1111/j.1365-2672.2009.04617.x

Hatoum, R., Labrie, S. and Fliss, I. (2012). Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. *Frontiers Microbiology 3(421)*, 1-12. https://doi.org/10.3389/fmicb.2012.00421

Ignot-Gutiérrez, A., Ortiz-Basurto, R.I., García-Barradas, O., Díaz-Ramos, D.I. and Jiménez-Fernández, M. (2020). Physicochemical and functional properties of native and modified agave fructans by acylation. *Carbohydrate Polymers 245*, 116529. https://doi.org/10.1016/j.carbpol.2020.116529

Liu, J., Willför, S. and Xu, C. (2015). A review of bioactive plant polysaccharides: Biological activities, functionalization, and biomedical applications. *Bioactive Carbohydrates and Dietary Fibre 5(1)*, 31-61. https://doi.org/10.1016/j.bcdf.2014.12.001

Lopez, M.G., Mancilla-Margalli, N.A. and Mendoza-Diaz, G. (2003). Molecular Structures of Fructans from *Agave tequilana* Weber var. azul. *Journal Agriculture Food Chemistry 51*, 7835-7840. https://doi.org/10.1021/jf030383v

Luo, M., Zhang, X., Wu, J. and Zhao, J. (2021). Modifications of polysaccharide-based biomaterials under structure-property relationship for biomedical applications. *Carbohydrate Polymers 266(February)*, 118097. https://doi.org/10.1016/j.carbpol.2021.118097

Mancilla-Margalli, N.A. and Lopez, M.G. (2006). Water-soluble carbohydrates and fructan structure patterns from Agave and Dasylirion species. *Journal Agriculture Food Chemistry 54*, 7832-7839. https://doi.org/10.1021/jf060354v

Miles, A.A. and Misra S.S. (1938). The estimation of the bactericidal power of the blood. *Epidemiology & Infection 38(6)*, 732-749. https://doi.org/10.1017/S002217240001158X

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry 31*, 426-428. https://doi.org/10.1021/ac60147a030

Miramontes-Corona, C., Escalante, A., Delgado, E., Corona González, R.I., Vázquez Torres, H. and Toriz, G. (2020). Hydrophobic agave fructans for sustained drug delivery to the human colon. *Reactive and Functional Polymers 146 (104396)*, 1-8. https://doi.org/10.1016/j.reactfunctpolym.2019.104396

Mitterdorfer, G., Kneifel, W. and Viernstein, H. (2001). Utilization of prebiotic carbohydrates by yeasts of therapeutic relevance. Letters in Applied Microbiology 33(4), 251-255. https://doi.org/10.1046/j.1472-765X.2001.00991.x

Mizrahy, S. and Peer, D. (2012). Polysaccharides as building blocks for nanotherapeutics. *Chemical Society Reviews 41(7)*, 2623-2640. https://doi.org/10.1039/C1CS15239D

Montañez-Soto, J., Venegas-González, J., Vivar-Vera, M., & Ramos-Ramirez, E. (2011). Extracción, caracterización y cuantificación de los fructanos contenidos en la cabeza y en las hojas del *Agave tequilana* weber AZUL. *Bioagro 23(3)*, 199-206.

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

Mueller, M., Reiner, J., Fleischhacker, L., Viernstein, H., Loeppert, R., & Praznik, W. (2016). Growth of selected probiotic strains with fructans from different sources relating to degree of polymerization and structure. *Journal of Functional Foods 24*, 264-275. https://doi.org/10.1016/j.jff.2016.04.010

Nava-Cruz, N.Y., Medina-Morales, M.A., Martinez, J.L., Rodriguez, R. and Aguilar, C. N. (2015). Agave biotechnology: an overview. *Critical Reviews in Biotechnology 35(4)*, 546-559. https://doi.org/10.3109/07388551.2014.923813

Nedovic, V., Kalusevic, A., Manojlovic, V., Petrovic, T. and Bugarski, B. (2013). Encapsulation systems in the food industry. In: *Advances in Food Process Engineering Research and Applications*, Pp. 229-253. Boston, MA. https://doi.org/10.1007/978-1-4614-7906-2

Pintor-Jardines, A., Arjona-Román, J.L., Totosaus-Sánchez, A., Severiano-Pérez, P., González-González, L.R. and Escalona-Buendia, H.B. (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. https://doi.org/10.1016/j.lwt.2018.03.060

Rodríguez-González, F., Parra-Montes de Oca, M.A., Ávila-Reyes, S.V., Camacho-Díaz, B.H., Alamilla-Beltrán, L., Jiménez-Aparicio, A.R. and Arenas-Ocampo, M.L. (2019). A rheological study of chicory and *Agave tequilana* fructans for use in foods. *LWT - Food Science and Technology 115(January)*, 108137. https://doi.org/10.1016/j.lwt.2019.05.035

Romano, N., Araujo-Andrade, C., Lecot, J., Mobili, P. and Gómez-Zavaglia, A. (2018). Infrared spectroscopy as an alternative methodology to evaluate the effect of structural features on the physical-chemical properties of inulins. *Food Research International 109(April)*, 223-231. https://doi.org/10.1016/j.foodres.2018.04.032

Santiago-García, P.A., Mellado-Mojica, E., León-Martínez, F.M., Dzul-Cauich, J.G., López, M.G. and García-Vieyra, M.I. (2021). Fructans (agavins) from Agave angustifolia and Agave potatorum as fat replacement in yogurt: Effects on physicochemical, rheological, and sensory properties. *LWT - Food Science and Technology 140(January)*. https://doi.org/10.1016/j.lwt.2020.110846

Velázquez-Martínez, J.R., González-Cervantes, R.M., Hernández-Gallegos, M.A., Campos Mendiola, R., Jiménez-Aparicio, A. and Arenas-Ocampo, M.L. (2014). Prebiotic potential of Agave angustifolia Haw fructans with different degrees of polymerization. *Molecules 19(8)*, 12660-12675. https://doi.org/10.3390/molecules190812660

Verma, D. K., Patel, A. R., Thakur, M., Singh, S., Tripathy, S., Srivastav, P. P.,... & Aguilar, C. N. (2021). A review of the composition and toxicology of fructans, and their applications in foods and health. *Journal of Food Composition and Analysis* 103884. https://doi.org/10.1016/j.jfca.2021.103884

Walz, M., Hirth, T. and Weber, A. (2018). Investigation of chemically modified inulin as encapsulation material for pharmaceutical substances by spray-drying. *Colloids and Surfaces A: Physicochemical and Engineering Aspects 536*, 47-52. https://doi.org/10.1016/j.colsurfa.2017.07.072.

Wu, X.Y. and Lee, P. I. (2000). Preparation and characterization of inulin ester microspheres as drug carriers. *Journal of Applied Polymer Science 77(4)*, 833-840. https://doi.org/10.1002/(sici)1097-4628(20000725)77:4<833::aid-app17>3.0.co;2-4