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ARTICLE

Coacervated Liposoluble Fructan-Based Host-Guest Microspheres as Unique Drug Delivery Materials

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A fundamental requirement in drug delivery design is the development of robust, target-specific, biocompatible, and pharmocokinetically-active cargo carriers. The use of natural polysaccharides for drug delivery has been a subject of long standing interest because they display many necessary attributes for such work. Herein, the current research details a new approach to a drug release system composed of microspheres derived from unique acetylated *Agave tequilana* Weber var. Azul fructans. The driving force and novelty behind this approach is that these fructans are liposoluble while still being able to be metabolized by *bifidobacteria* (lower GI, native colon bacteria). Modification of fructan solubility through acetylation supported the preparation of microspheres by precipitation-coacervation, a new synthetic approach for these polysaccharides that facilitated the encapsulation of liposoluble cargo molecules such as Ibuprofen. It was found that the enzymatic activity of *B. animalis*, a representative bacteria found in the human colon, was reduced, albeit, but not at the expense of providing a very compellingly favorable drug release profile.

Key Words: *Fructans, Agave tequilana Weber var. Azul, acetylation, bifidobacteria, fermentation*

INTRODUCTION

Fructans or inulins are plant reserve carbohydrate polymers that at the biomolecular level consist of fructosyl residues as the monomeric units with a single sucrose (fructose-glucose) residue usually found at the terminus. They are found both in monocotyledons and dicotyledons and were first isolated from *Inula helenium* which is why they are otherwise known as inulin. Inulin is a β-(2→1) linear fructan constructed from 1-kestose; however, other types of fructans such as those found in grasses are β-(2→6) biomacromolecules with building units based on 6-kestose. Fructans, like all polysaccharides, are rich in hydroxyl units. Each fructose ring generally accommodates three (3) pendant (free) hydroxyls that are located on the C_2 (2°), C_3 (2°), and C_5 (1°) atoms.

Only a few plants contain fructans in high abundance, *viz.*, root of chicory (*Cichorium intybus var. sativum*, an excellent coffee substitute) and the tubers of Jerusalem artichoke (*Helianthus tuberosus*, species of sunflower that are native to E. North America). Another rich source of fructans is the blue agave plant (A. *tequilana Weber* var. azul). Agave plants are succulents that belong to the lily family where in Mexico, more than 136 species of agave grow with 26 subspecies, 29 varieties, and 7 types. Blue agave is traditionally used to prepare the world renowned Mexican spirit "tequila." More than 80 wt-% of the carbohydrate content in blue agave originates from its fructan content. Fructans from *A. tequilana* Weber var. azul, however, are strictly not inulins as previously thought. Inulin is a *linear* (2→1) linked fructose polymer capped by a sucrose unit having an average degree of polymerization (DP) from 2 to 60, depending on the plant. Fructans, on the other hand, are a complex mixture of fructo-oligosaccharides containing principally β-(2→1) linkages, but in addition, they possess β-(2→6) linkages and branch residues [1-3].

Currently, fructans from blue agave have increasingly been marketed as dietary supplements, a source of fructose, and drug excipients [4]. These commercial fructan varieties have been mistakenly marketed as *inulin*, although this name indicates that the polysaccharide is linear. The opportunity to manipulate their unique structure and chemical characteristics for advanced biomedical applications (*e.g.*, functional composites, tissue engineering, drug delivery) is ripe, but few efforts have been demonstrated as of late [5-7]. The field of

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bio-based materials for biomedical applications, in general and especially for drug delivery, has not received as sufficient attention as its biocompatibility warrants [8-14].

In general, orally administered controlled release drugs typically do not possess sufficient properties to lend them as successful drug release candidates within the gastro-intestinal (GI) tract [15, 16]. The colon is ideal for both systemic and local drug delivery where high concentration distribution of drugs can be allowed by a colon-targeted drug delivery approach. The colon offers definite therapeutic benefits because it displays near neutral pH and has a long transit time. However, reaching the colon is difficult – a dosage formulation must account for various chemical obstacles in the GI tract, i.e., protection of the drug from hydrolysis which adversely results in premature release in the acidic stomach as well as account for water solubility [17, 18]. Although a number of polysaccharide candidates have been extensively studied to date, fructan-based systems demonstrate a pronounced GI appeal because they have been able to resist hydrolysis in the upper GI and can be subsequently fermented by colonic microflora. However, to overcome their unfavorable water solubility, substitution is an approach that has been found to be effective. Vervoort formulated methylated inulin hydrogels for colonic drug delivery and found that parameters such as the degree of substitution, feed concentration, concentration of the polymerization initiators, pH, and ionic strength are critical to swelling and effective drug release [19]. In a follow up study, Vervoort studied the *in vitro* enzymatic digestibility of inulin hydrogels by inulinase enzymes from a strain of *Aspergillus niger* and discovered that the enzymes can diffuse into the hydrogels to degrade them [20].

The current focus of this research, therefore, was to evaluate the potential of a modified fructan system for sustained release of a model drug (Ibuprofen) through enzymatic (*bifidobacteria*)-mitigated degradation. The heart of the approach was the preparation of microspheres from fructans by means of a simple precipitation-coacervation step. First, the fructans were modified by acetylation to different degrees (up to nearly three due to three free hydroxyls/fructosyl unit) to enhance their O/W partitioning coefficient, *i.e.,* decrease their water solubility while consequently increasing their liposolubility. This was done to increase their stability under acidic conditions (*i.e.*, in the stomach) and also improve their response to simulated hydrolysis in the colon. Next, the metabolic response to the fructan microspheres containing Ibuprofen was studied to determine if *bifidobacteria* (colon bacteria) would hydrolyze the acetylated fructans and, if so, what would the drug release profile look like.

Materials And Methods

Materials

Fructans Fructans were isolated and purified from the juice of *Agave tequilana* Weber var. azul according to established procedures [4, 21]. Briefly, small sections of agave were ground in a Bauer refiner (Bauer Bros Co, Canada) and extracted with hot water in a spin basket. Proteins were separated from the supernatant by thermal flocculation at 80 °C for 40 minutes.

The solution was filtered over a bed of diatomaceous earth, purified and clarified by treatment with activated carbon, and finally demineralized and clarified completely through ionic exchange columns. The solution thus obtained (1 L, 22 ° Brix) was dried in a Yamato spray-drier model ADL31 (Orangeburg, NY), to obtain fructan powder. In addition, commercial agave fructan (inufib batch 4IIPP12336, Idea Company) was used for fermentation.

Bacterial strains for fructan fermentation The following strains were obtained from the American Type Culture Collection Rockville, Md (ATCC) collection and freeze-dried: *Bifidobacterium adolescentis* ATCC 15703 and Raff S.A de C.V., strains: *Streptococcus chloracidobacterium* Y470, *Bifidobacterium animalis* BGP93 and *Lactobacillus acidophilus* LA3.

Reagents for synthesis of fructan derivatives Acetic anhydride, 97% (Golden Bell reagents, Sparks, NV); N, N-dimethylformamide (Fermont, Canada); sodium acetate (Chemical products, Monterrey); Acetone, analytical grade and ethanol, 96% (Analityka, Mexico); Tween 80 (Fluka); Ibuprofen (acid R, S-2-(4-Isobutilfenil) propionic acid) (Sigma Aldrich, Milwaukee).

Methods

Fructan characterization The degree of polymerization and molecular weight of the agave fructans were determined by Size Exclusion Chromatography (SEC) and proton Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR).

Fructan esterification Fructan acetylation was carried out according to previously established procedures [2, 16]. Briefly, the esterification was conducted in a 3-necked round bottom flask at 40 °C with constant agitation under a nitrogen blanket. First, fructan (1 g) was dissolved in dimethylformamide (7 mL), and then acetic anhydride was added drop-wise (10.3 mL, 106 mmol) with sodium acetate as the catalyst (0.05% total volume). Several fructan esters products were obtained by varying the reaction time. The solubility of the esterification products was tested in different solvents. The substitution degree was calculated in all cases by ^{1}H-NMR (300 MHz). The fructan esters were also characterized by Infrared spectroscopy (FTIR – ATR) and ^{1}H-NMR.

Microsphere preparation Microspheres were prepared according to the literature [2]. Briefly, the acetylated water-soluble fructans were prepared by dissolving a sample of 7.5 mg in 300 µL of water and pouring the solution drop-by-drop into 40 mL of ethanol-acetone (50:50, v/v) containing Tween 80 (0.5%, w/v) at room temperature under constant agitation [22, 23]. The resultant microspheres were recovered from the supernatant by freeze-drying. Microspheres from acetylated fructans with a substitution degree of ~ 3 were prepared by dissolving 7.5 mg of fructans into 300 µL of acetone. The solution was poured into 40 mL of water containing Tween 80 (0.5%, w/v) at RT under vigorous stirring. The suspension was allowed to stir for 2 hours, after which the microspheres were recovered by centrifugation and freeze-drying.

Ibuprofen-loaded microspheres The encapsulation of Ibuprofen into the microspheres was carried out by a procedure similar to microsphere preparation, except that three different quantities of Ibuprofen (1, 5, and 10 mgs) were dissolved in tandem with fructans (a quantity of 7.5 mg) into 300 µL of acetone before precipitation in water. The size and morphology of the microspheres were analyzed using a FE-SEM (JEOL JSM-6400F) whose images were deconvoluted by Revolution SEM software.

Selection of fermentative conditions for microorganism growth Four different culture media reported in the literature were used: synthetic medium (SM) [24]; modified synthetic medium (MSM without salts); *Lactobacillus*-modified medium (LBM) which is a half MRS DIFCO BD medium (Becton, Dickinson and Co.) for *Lactobacillus* strains specifically modified to use 10 g/L of glucose concentration; and lastly, a medium for *Bifidobacteria* (BFM) [25].

The strains were grown anaerobically within a volume of 80 mL at 37 °C. Growth was monitored by measuring the increase in optical density (OD) at λ= 620 nm as determined by a UV-VIS spectrophotometer. Samples from the inoculum (5 mL) were obtained and transferred for activation in vials with different culture media and different carbon sources. Once the culture media containing the microorganism was activated, samples of 3 mL were periodically taken and their absorbances were recorded. First, a sample was taken at time zero (start of fermentation). Subsequently, samples were obtained at 0.5 and 1 h; afterwards, samples were taken every 2 hours up to and including 12 hours of fermentation. Next, samples were obtained every 3 hours up to and including 72 hours of fermentation. The culture media that displayed the highest growth of microorganisms as indicated by OD was selected to further evaluate the effect of pH and the source of carbon (oligosaccharides and monomeric sugars) on the growth of bacteria. Finally, fermentation *in vitro* assays were conducted using fructan microspheres as the carbon source at a concentration of 10 g/L with and without (control) Ibuprofen.

HPLC compositional analysis of carbohydrates and short chain fatty acids (SCFA) The molecular composition of the carbohydrates present in all of the samples was obtained by a Waters HPLC equipped with an RI detector (Waters 2410) and an analytical column for carbohydrates (BioRad HPX-87 C – 300 × 7.8 mm). The analytical conditions that were used were the following: 60 °C; a mobile phase of HPLC grade water at a flow rate of 0.5 mL/min with sample injections of 40 µL. The short-chain fatty acids (SCFA) from fermentation were analyzed likewise, except an HPLC column for ionic exclusion was used (BioRad HPX – 87 H (300 × 7.8 mm). The analytical conditions that were used were the following: 50 °C; a mobile phase of HPLC grade water and 4 mM sulfuric acid at a flow rate of 0.5 mL/min with sample injections of 40 µL [26]. Esterification, fermentation, metabolite production, and ibuprofen release experiments were carried out with at least three replicates.

RESULTS AND DISCUSSION

Native fructan characterization

SEC analysis of the isolated fructans revealed that the agave fructan is a polydisperse carbohydrate with an M_w 2,600 Da and an M_n 1,600 Da (M_w/M_n = 1.6) with an average chain length consisting of ~ 14-16 fructose units. The anomeric carbon of the glucose unit at the reducing terminus of the fructosyl chains was identified at 5.38 ppm (H-C*) by ^{1}H-NMR [27]. Signals in the 3.5-4.3 ppm region corresponded to the C-Hs in the skeletal framework of the remainder of the structure. The degree of polymerization (DP) was more or less corroborated by the ^{1}H-NMR calculated value of 15, which in general based on the SEC and NMR data, corresponds to one glucose unit per ~ 14 fructose units. Although a narrow polydispersity favors a more homogenous modification of the material, a polydispersity of 1.6 provides a range of molecular weights that likely encourage better growth adaptation of the microorganisms because of the M_w variations of the feedstock.

Acetylated fructans

The acetylation of Agave fructans was a key chemical step intended to obtain generally soluble fructans in lipophilic media. Thus, their insolubility in water would facilitate the formation of microspheres through coacervation. FT-IR spectra were afterwards collected to authenticate acetylation. They showed a diminished OH group stretching band at ~ 3353 cm^{-1} and a new band corresponding to a carbonyl group (C=O, 1745 cm^{-1} stretching frequency) of an ester linkage that was absent in the native fructans. In addition to the carbonyl functional group signature, acetylated fructans also showed characteristic signatures from the acetate C-O stretching band at 1230 cm^{-1} and 1370 cm^{-1} from the acetate -CH_3 group bending. These results are in agreement with what has been reported for inulin acetate [22, 23] and Agave fructan acetate [2].

The ^{1}H-NMR spectrum of a native fructan is shown in Figure 1. The carbon protons (C-H) appear between 2.8 and 3.7 ppm, whereas the protons from the hydroxyl groups are located between 4.2-4.9 ppm. Protons from the anomeric carbon at the reducing terminus of the fructan oligosaccharide are visible as a broad singlet between 5-5.4 ppm.

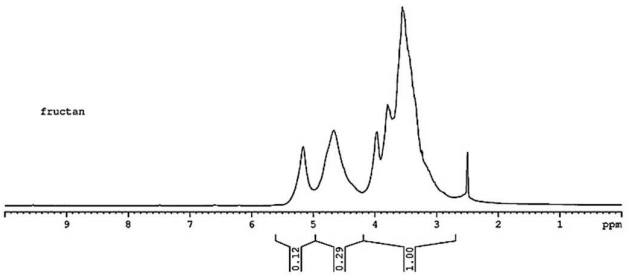

Figure 1. ^{1}H-NMR spectrum of fructan in DMSO-d[6].

The ^{1}H-NMR spectrum of acetylated fructan revealed the presence of methyl singlets at ~ 1.93 ppm arising from the acetate (-$COCH_3$) methyl groups absent in the native fructans. This region closely corresponds to the range of C-H protons in the native fructan. By comparing the integration of the signal from the seven protons in the fructose unit (2.8 and 4.2 ppm) to the area of the three protons

of the CH_3 group of the alkyl chain (at approximately 1.9-2.2 ppm), the degree of substitution obtained by the acetylation reaction was obtained [28]. As previously described, the degree of acetylation for the fructans can be controlled. Figure 2 illustrates the degree of hydroxyl substitution (acetylation) over a broad window of reaction times. The time scale shown is indeed broad enough to provide controllable degrees of substitution. As expected from steric arguments, the curve is characterized by a distinct exponential pattern where one degree of substitution proceeds much more rapidly than the ensuing substitution, particularly up to full substitution (DS = 3).

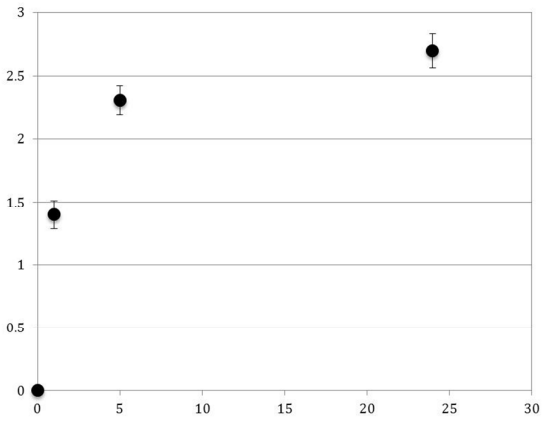

Figure 2. A plot of the degree of acetylation as a function of time for Agave fructans.

Microspheres

The next part of the work was to develop microspheres possessing different degrees of acetylation and study how they affected hydrolysis and enzymatic activity. Past work with inulins has shown that acetylated inulins having degrees of substitution from 1.6 to 2.8 are completely water insoluble, but highly soluble in many organic solvents [29]. Therefore, it was possible to synthesize acetylated agave fructans (AF) with a DS of 1.4 that were as a result of the acetylation serendipitously amenable to coacervation. They were developed into microspheres by solvent precipitation using Tween 80 as a dispersant that were then freeze-dried and shown to have average diameters (from SEM) ranging from 0.5 to 5 µm.

Encapsulated microspheres were prepared using the same procedure, except for the inclusion of guest molecules (in this case, Ibuprofen) in the original acetylated fructan solution. Ibuprofen was chosen as a representative guest molecule because of its availability, common usage, and to enhance targeted delivery (gut) and accompanying release profile. Samples of fructans (7 mg, DS 2.7) were mixed with Ibuprofen at various concentrations after which microspheres with larger average diameters relative to the control microspheres of 5 to 35 µm were observed. It was also observed that higher loadings of Ibuprofen gave rise to microspheres that were larger and more uniform in diameter likely arising from a greater turgor pressure exerted by the higher guest loading. In particular, the microspheres made with the highest loading in this study (10 mg) were quite uniform and smooth (Figure 3). The differences in size and shape obtained with the microspheres very likely resulted from the differences in the partition coefficient of the produced material, range of molecular weights, different degree of acetylation (DS reported is an average), and the topochemistry of the reaction. As it was noted before, this could be an advantage for the growth of bacteria and subsequent release of cargo from the microspheres.

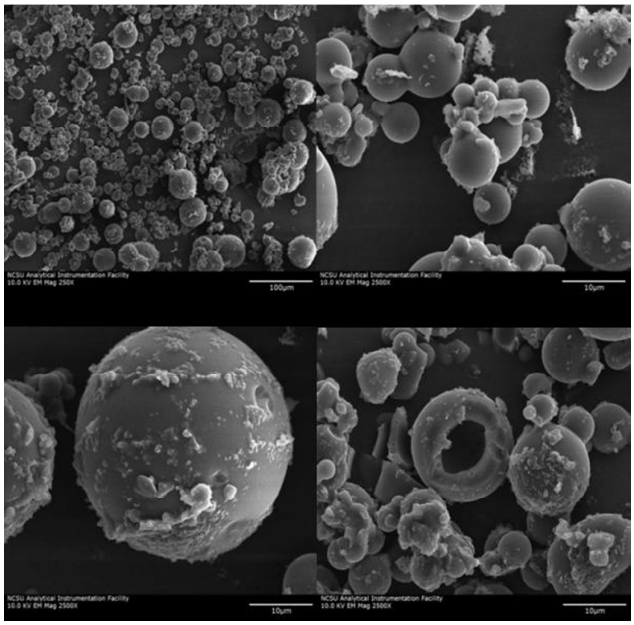

Figure 3. SEM microphotographs of highest Ibuprofen-loaded fructan-based microspheres obtained by coacervation.

Biomimetic studies: *bifidobacteria* growth in the presence of the fructan microspheres

The following fermentation experiments were run to study enzymatic activity and ensuing microsphere hydrolysis. A number of different fermentation parameters were screened including the culture media, microorganisms, pH, and initial substrate concentration to optimize the hydrolytic activity of the system against the fructans used. After a number of screenings, a *Lactobacillus*-modified medium (LBM) at pH 6.5 and initial fructan concentration of 10 g/L with the bifidobacteria known as *B. animalis* was determined to be optimal to degrade the specific fructans used in our study. Subsequently, the growth of *B. animalis* was evaluated in LBM with native fructans (NF), acetylated fructans (AF1.4 and AF2.7 having DSs of 1.4 and 2.7, respectively), and microspheres prepared using AF1.4 (unloaded, UM). Figure 4 illustrates that *B. animalis* has a higher growth with native fructans as a carbon source, while a higher DS (acetylated fructans) resulted in a lower growth, likely due to the presence of acetyl moieties. The difference between the growth at DS of 1.4 and 2.7, however, is very pronounced and extremely useful. It demonstrates that even at a DS = 1.4, the fructan microspheres allow for bacterial activity;

namely, they can be hydrolyzed and used as an energy source, thus allowing any encapsulated guest molecules to escape! Indeed, there does not appear to be a similar finding in the literature. In fact, past work has shown that modified (acetylated DS = 1.6, and methylated DS = 2.4) inulins exhibited virtually no susceptibility to enzymatic activity (in Fructozyme®, a mixture of exo- and endo-inulinase enzymes from a selected strain of *Apergillus niger*) [29]. In that previous study, it was shown that the insolubility of the acetylated inulin was *not* the reason for its resistance to enzymatic hydrolysis, but it was due to the conformational/structural changes that resulted from derivatization. Despite further experiments in which acetylated inulin was solubilized in a 50:50 mixture of DMSO/water (interestingly, continued enzymatic activity with native inulins was shown despite the organic composition of the medium), it was found that both it and methyl inulin (soluble in water) were still *not* susceptible to enzymatic activity. Similar past modification studies with acetylated dextran were done that indicated the resultant polysaccharide, despite being water soluble, was *not* degraded by bifidobacteria, again owing to conformational changes in the molecule from derivatization [30]. Remarkably, the acetylated fructans with DS = 1.4 used in this study (as is or coacervated) still allowed for pronounced enzymatic activity. Although the fructans in this study are slightly different than inulins, a related study had already been done on native inulins having a similar DP (14) to the ones used in this study and showed excellent bifidobacteria activity [31]. It appears that the current system is sufficiently balanced in terms of water insolubility and structural characteristics to maintain acid stability (as is the case with all fructans/inulins) while allowing for enzyme accessibility at the target despite acetylation. Such a result portends its potentially high utility as a cargo carrier to the gut.

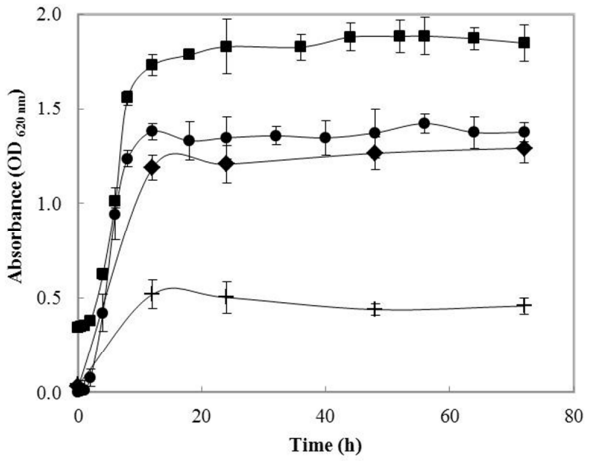

Figure 4. *B. animalis* growth in fructan and acetylated fructan as observed by the increase in the optical density at 620 nm in LBM at pH 6.5. NF (■): native fructan; AF1.4 (●): acetylated fructan DS 1.4; AF2.7 (+): acetylated fructans DS 2.7; UM (◆): unloaded acetylated fructan microspheres DS 1.4.

Fructan degradation and metabolic activity

The components of culture media have an essential role on microorganism growth because they provide nutrient (carbon) source for the synthesis of metabolites and for cell maintenance. When they adequately support the functioning of the microorganism, the culture medium (LBM in this case) contains short chain fatty acids (SFCA), a clear signature of microbial metabolic activity arising from digestion of the nutrient (e.g., fructans/inulins). Figure 5 shows degradation of fructan and fructan derivatives as wt-% fructan consumed, and the production of acetic and lactic acids (g/L) at the end of fermentation. The substrate that was degraded to the highest extent (75.89 wt-%), not surprisingly, was NF, and hence can be considered an excellent food source for the bacteria; whereas, as expected, acetylated fructans were degraded to a lower extent, in a manner consistent with the substitution pattern of acetyl groups (AF $DS_{2.7}$ was the least degraded). Such a result is consistent with the logarithmic (absorbance) data for the growth of the microorganism as shown in Figure 4, i.e., the higher the DS, the lower the lower the degradation of the fructan and hence the growth of the organism. The fact that the unloaded acetylated microspheres with DS = 1.4 were degraded to nearly half that of the native fructans despite being acetylated is a remarkable finding.

Based on the fructan consumption (as shown in Figure 5), production of SCFA was expected to be higher for native fructan. This was the case, especially for production of lactic acid, in which it was three (3) × the amount of acetic acid (0.85 g/L) produced. Reduced amounts of acetic acid were found because it is typically used in other metabolic pathways. The acetic acid : lactic acid ratio was higher in acetylated fructans, not surprisingly, because of acetic acid release that originates from the deacetylation of the acetyl moieties. Finally, and not surprisingly, Ibuprofen-loaded microspheres were much less degraded relative to the native fructan, but at a rate acceptable for biomedical applications because it is known that many host-guest drug release profiles generally follow a very rapid release profile in which 50-80% of cargo release occurs within the early phase of hydrolysis. It is likely that acetylation in combination with Ibuprofen worked in synergy to reduce the digestive activity of the microorganisms. Ibuprofen is known to exert an inhibitory effect on bacterial digestion during release because of the acidity it generates upon dissolution. Future studies will consider much less pharmaceutically active guest molecules to probe their effect on microbial digestion.

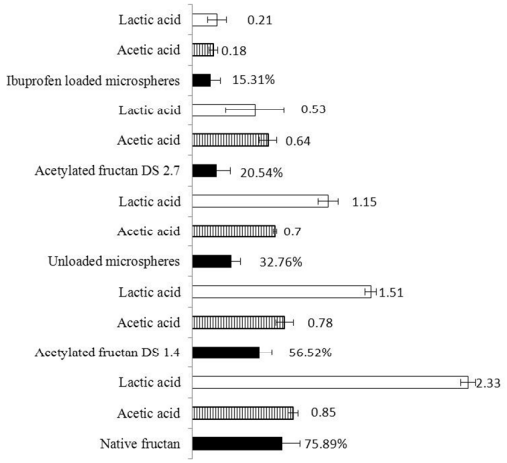

Figure 5. Fructan consumption (wt-%) and concomitant production of short chain fatty acids (SCFA) (g/L) from the enzymatic activity of *B. Animalis*.

Microsphere loading and release profile of *Ibuprofen*

Ibuprofen ($C_{13}H_{18}O_2$) is a globally available and safe nonsteroidal anti-inflammatory and analgesic medication that is used to relieve inflammation, pain, and fever. However, its low solubility and short half-life lead to a low bioavailability and may have adverse effects. Thus, a number of efforts have been undertaken to address these deficiencies. One of the principal approaches is to encapsulate it within an excipient (delivery system) to improve bioavailability and alleviate any adverse effects. Thus, in this study, the coarcevates developed may provide a potential route to improve solubility, stability, and bioavailability. We therefore proceeded to obtain Ibuprofen release profiles to biomimetically simulate its release in the gut. Microspheres that were loaded with Ibuprofen were subjected to fermentation with *B. animalis* during which samples were taken every 12 hours over a total fermentation window of 72 hours. The released Ibuprofen was analyzed by HPLC and the results are shown in Figure 6.

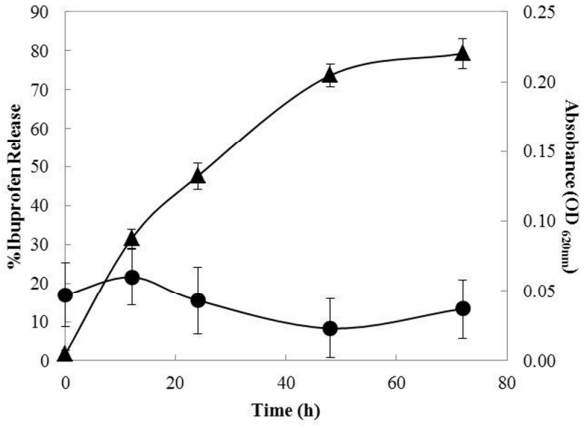

Figure 6. Profile of %-age of Ibuprofen released from acetylated fructan microspheres during fermentation of B. *animalis* where solid triangles (▲) represent "% Ibuprofen Released" and solid circles (●) represent "Absorbance (OD_{620nm})".

It was found that coacervation allowed for the preparation of microspheres that encapsulated as much as 33% of the Ibuprofen loaded which is in general an excellent result when using such an approach. In contrast, in recent studies attempting to encapsulate it in similarly innocuous materials (clays), less than 15% of the Ibuprofen loaded was uptaken [31]. The release profile shown in Figure 6 represents the pinnacle result for the current study. It is shown that over a biologically relevant 2-3 day window for the release of these types of drug (non-steroidal anti-imflammatory drugs, NSAIDs), Ibuprofen was released at not only a very regular rate of ~ 0.2 mg/hr, but up to ~ 80% total load release. This result is quite favorable in comparison to a similar study where gellan gum hydrogels were used and showed 50% reduced release rates and a shorter regular sustained release windows (5 hours, but total release at 25 hours) [32].

The growth of bacteria during fermentation increased during the first 10 hours of fermentation, in agreement with the enzymatic digestion of the fructan microspheres as already described in our earlier results, after which the bacterial growth decreased and leveled off, also in accord with what was found earlier. This attenuation in bacterial grown as monitored by the absorbance at 620 nm is likely due to an inhibitory effect arising from the acidic environment produced by Ibuprofen that is not favorable for bacterial growth and consequent production of enzymes [33, 34]. Interestingly, the decline in the enzymatic activity after the first 10 hours does not appear in any way to affect the drug release profile to any great extent. It appears as stated earlier that sustained enzymatic activity over this short window is sufficient to modify the morphology of the microspheres to provide a very even and sustained release profile over the next ~ 60 hours.

Conclusions

It has been shown in this biomimetic degradation study of fructans that bifidobacteria can digest acetylated *Agave tequilana* fructan microspheres as a carbon source during fermentation *in vitro* thus allowing for the escape of guest molecules such as Ibuprofen. The fructans can be acetylated to higher degrees of substitution (up to 2.7) to reduce their solubility in water and increase their solubility in lipophilic systems. The bifidobacteria were able to consume up to 75% of native fructan, while the degradation could be modulated to nearly one quarter at a DS = 2.7. The utilization of a simple coacervation procedure allowed the preparation of acetylated fructan microspheres that could be loaded up to 33% with Ibuprofen in an excipient drug model approach. Drug release profiles for Ibuprofen from the microspheres were accomplished by fermentation with *B. animalis*, a typical gut bacterium. It was found that a maximum release of Ibuprofen was obtained after 12 hours of fermentation that corresponded to a maximum bacteria growth. Afterwards, the concentration of Ibuprofen was nearly constant over the ensuing 60 hours of fermentation. These results compare very favorably to the literature with respect to a liposoluble, stable,

and targeted drug delivery agent susceptible to bifidobacterial degradation.

Acknowledgement

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