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Fructans (agavins) from *Agave angustifolia* and *Agave potatorum* as fat replacement in yogurt: Effects on physicochemical, rheological, and sensory properties

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ABSTRACT

In this work, agavins (agave plant fructans) potential as fat replacer in dairy products was evaluated. Yogurt non-fat and fat reduced samples were prepared, agavins from *Agave angustifolia* and *A. potatorum* species were added to fat reduced products to evaluate their effect on the physicochemical, rheological, and sensory properties in the final products. Significant differences in the viscosity, syneresis and water retention capacity were observed among samples. All yogurts exhibited characteristics typical of a weak viscoelastic gel (G'>G'') and strain-thinning behavior. Physicochemical and rheological differences were strongly related to the agavins amount in yogurt fat-reduced samples. Agavins did not impact sensory attributes such color, smell, and taste in fact; they improved mouth feel and texture. HPAEC-PAD profiles demonstrated that agavins remain unaffected along the yogurt elaboration process. FTIR spectra showed remarkable differences on all final products composition, been more evident on fatty acids and carbohydrates regions. FTIR-PCA models allowed samples classification among treatments and samples discrimination according to the agavins content. Agavins addition to dairy products has a great potential as functional ingredient in the manufacture of low-fat, prebiotics and symbiotic products. Finally, compiled information increases our knowledge on the broad technological functionality that agavins may have in other food industry niches.

1. Introduction

Achieving and maintaining good health is a major goal of consumers today; hence, the consumption of nutraceutical and/or functional foods has increased, reflecting the critical importance of diet and lifestyle on health. A nutraceutical is a food or part of a food that produces health benefits including the prevention and/or treatment of a disease. A functional food is any food or ingredient that has a positive impact on an individual's health, physical performance, or mental state, in addition to its nutritive value (Santini and Novellino, 2014). Both nutraceuticals and functional foods have been recognized for their potentially major impact as preventive and/or therapeutic tools, in addition to their function as a source of macro and/or micronutrients that are necessary for all metabolic and body functions (Santini & Cicero, 2020).

Therefore, the combined and concerted action of nutrients and biologically active compounds is a known indicator of a nutraceutical food (Durazzo, Lucarini, & Santini, 2020). In this context, dairy products are the food matrices most commonly supplemented with probiotic ingredients and prebiotics, as they offer the easiest delivery of probiotic microorganisms to the human intestine. The technological processes of cheese and yogurt are advantageous for their supplementation with probiotic cultures and prebiotic ingredients such as inulin (Karimi,

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Azizib, Ghasemlouc, & Vaziri, 2015; Karimi, Mortazavian, & Amiri-Rigi, 2012). Yogurt is one of the most popular fermented products throughout the world owing to its dsnutritional and therapeutic properties, and its status as a good source of probiotics. *Lactobacillus acidophilus* and *Streptococcus* are the main probiotic bacteria employed in the yogurt manufacture, and confer several health benefits, such as improved digestion, alleviation of the symptoms of lactose intolerance (Akolkar, Sajgure, & Lele, 2005; Sanders, 2000), and production of exopolysaccharides essential for product texture (De Vuyst, De Vin, Vanigelgem, & Degeest, 2001).

The presence of fat in dairy products has a considerable impact on the physical properties, such as rheological characteristics, texture, and microbiological stability. Low-fat foods are an option for consumers interested in reducing the risks related to metabolic syndrome diseases and/or maintaining a healthy physical condition. However, low-fat yogurts are predominantly unacceptable to consumers because of their rheological characteristics and increased syneresis. These effects can be diminished by the addition of some carbohydrates, such as fructans, which are classified as dietary fiber (U.S. Department of Health and Human Services, 2018) with prebiotic capacity and are considered generally recognized as safe (GRAS) by the FDA (Franck, 2002); consequently, the addition of these to yogurt does not affect the safety of the food matrix produced. Inulin-type fructans have been used widely as a fat substitute in low-fat dairy products as they can form microcrystals when mixed with water or milk. These crystals are not perceived in the mouth, but interact with the food matrix forming a fine creamy texture that produces a sensation more comparable with that of full-fat milk (Kalyani, Kharb, & Thompkinson, 2010).

Agavin-type fructans are soluble carbohydrates that can be used to incorporate or increase a fat-mimetic effect in some foods. As fructans, agavins are nondigestible carbohydrates with a highly branched structure containing β(2–1) and β(2–6) bonds (Mancilla-Margalli & López, 2006; Mellado-Mojica & López, 2012). Likewise, its food safety has been demonstrated and it is classified as a dietary fiber (Carabin et al., 1999; U.S. Food and Drug Administration Center for Food Safety Applied Nutrition, 2020). Consequently, they reach the large bowel mostly unchanged and are suitable for fermentation by the colonic microbiota, producing short-chain fatty acids, such as acetic, propionic, and butyric acids, which are attributed to many benefits, including weight loss, increased secretion of hormones involved in the regulation of food intake (Santiago-García & López, 2014), and improved absorption of bone minerals such as calcium and magnesium (García-Vieyra, Del Real, & López, 2014). In addition to these health-beneficial effects, agavins have interesting properties that are linked to their degree of polymerization (DP) (Santiago-García, Mellado-Mojica, León-Martínez, & López, 2017).

Indeed, one of the current market trends is the production of dairy products with a reduced fat content, with the aim of minimizing the negative effects of lipids on consumer diets. The effect of agave fructans on some physical characteristics of dairy products has been studied previously by Crispín-Isidro, Lobato-Calleros, Espinosa-Andrews, Alvarez-Ramírez, & Vernon-Carte (2015); they showed that the addition of inulin to yogurt samples formed gels with secondary structures between casein micelles, whereas agavins tended to deposit themselves on the surface of casein micelles, increasing the sensory attributes of those treatments with fructans. Palatnik, Aldrete Herrera, Rinaldoni, Ortiz Basurto, and Campderrós (2016) reported the texturizing role of fructans in cheese, obtaining a creamy cheese with good protein retention and low fat content without significant differences to control samples in other sensory aspects. Topolska, Filiak-Florkiewicz, Florkiewicz, & Cieslik (2017) showed the effect of Jerusalem artichoke fructans with different DPs on the stability of strawberry sorbet stored for up to 22 weeks. Given the economic importance of agaves in Mexico, their great biodiversity, and the recently reported potential of their fructans as a prebiotic, the objective of this work was to evaluate the effect of agavins with a large degree of polymerization (LDP) from *Agave angustifolia* Haw. and *Agave potatorum* Zucc. on the physicochemical, sensory, and rheological properties of low-fat yogurt supplemented with different percentages of these fructans.

2. Materials and methods

2.1. Agave plant harvest and agavins extraction

Eight-year-old *A. angustifolia* Haw. and 6-year-old *A. potatorum* Zucc. plants were harvested in Totolapan and Sola de Vega in Oaxaca, Mexico. Following collection, the agave stems were milled for 30 s at 10 000 rpm, and the juice and fiber were recovered. Fructans were extracted according to the protocol of Mellado-Mojica and López (2012) with some modifications. Briefly, 100 g of agave fiber was extracted twice with 100 mL 80% ethanol with continuous stirring for 1 h at 55 °C. The sample was filtered, the supernatants were combined, and the filtered agave juice was concentrated by evaporation. The resulting solution was clarified through activated carbon, diatomaceous earth, and an ion exchange resin to eliminate calcium and chelates. Agavins were separated by their solubility; agavins with a short degree of polymerization (SDP) remained in the alcoholic solution and those with a LDP precipitated and were more viscous. The last fraction was spray-dried and stored in a humidity-free container until use.

2.2. Yogurt samples

2.2.1. Yogurt manufacture

Six different yogurt samples were prepared. The sample nomenclature is listed in Table 1. First, 15 g of sucrose was placed in a 500 mL Erlenmeyer flask containing 250 mL of non-fat-reduced or fat-reduced milk according to Table 1; then, the agavins (7.5 g and 15 g, for 3% and 6%, respectively) were added to the flasks, and all ingredients were homogenized and dissolved using a magnetic stirrer for 5 min. The mixtures were pasteurized at 83 °C for 15 min and cooled to 45 °C in a cold-water bath, when inoculation was performed (30 $mg \cdot L^{-1}$ of lactic Liofast SACCO). For fermentation, the samples were placed in an incubator (red LINE, model RE 53-UL) at 45 °C until an acidity of 78°D (4–5 h) was reached. Yogurt samples were cooled and then refrigerated (4 °C ± 1 °C) for 24 h. Prior to use, the yogurt samples were removed from the refrigerator and gently stirred.

2.3. Physicochemical properties of yogurts

2.3.1. Fat content, acidity, and total solids content in prepared yogurts

The fat content in the yogurt samples was determined using Butyrometer Gerber – Sichler for skim milk; titratable acidity was expressed as g of lactic acid per 100 mL of milk; and the total solids content was determined by oven drying (AOAC, 2016) after 3 days after preparation.

2.3.2. Syneresis

Syneresis, a yogurt stability parameter, was measured in samples stored refrigerated for 11 days in according with the protocol described by Keogh and O'Kennedy (1998), with some modifications. In brief, 10 g of yogurt stored at 4 °C ± 1 °C was placed in conical tubes and centrifuged (HERMLE, model Z 323 K) at 1800 rpm for 10 min at 4 °C ± 1 °C.

Table 1
Nomenclature and composition of yogurt samples.

Sample Nomenclature	Sample description
YNFRC	Yogurt Non Fat-Reduced Control
YFRC	Yogurt Fat-Reduced Control
YFR_AA3%	Yogurt Fat-Reduced *Agave angustifolia* agavins 3%
YFR_AA6%	Yogurt Fat-Reduced *Agave angustifolia* agavins 6%
YFR_AP3%	Yogurt Fat-Reduced *Agave potatorum* agavins 3%
YFR_AP6%	Yogurt Fat-Reduced *Agave potatorum* agavis 6%

The clear supernatant was poured, weighed, and expressed as a percentage of weight relative to the original weight of the yogurt. All analyses were performed in triplicate.

2.3.3. Water retention capacity

The water retention capacity in yogurt samples was estimated according to the method if Lalou, Kadri, and Gkatzionis (2017). Aliquots of approximately 20 g of yogurt were centrifuged for 10 min at 1250×*g* at 4 °C. The supernatant whey was recovered in a clean tube and weighed. The water-holding capacity was calculated as the difference between sample and the whey weight, and expressed as a percentage (g/100 g).

2.4. Rheological measurements

2.4.1. Brookfield viscosity

The viscosity of the yogurts was measured at 25 °C with a Brookfield model RVF viscometer, using the number 2 needle at a speed of 50 rpm for all samples. The compiled data were reported in centipoise (cP) (Aryana, Plauche, Rao, Mcgrew, & Shah, 2007).

2.4.2. Rotational and oscillatory shear measurements

Yogurts samples were analyzed at 1 and 11 days after preparation. Yogurt samples were gently stirred 10 times with a tablespoon before the rheological analysis and all measurements were performed at 10 °C and in triplicate.

A stress-controlled rheometer (model Physica MCR301, Anton Paar, Austria) with parallel plate geometry (diameter, 50 mm; gap height, 2 mm). The plate contact surface was covered with a 180 grit silicon carbide sandpaper with a mean particle diameter of 82 μm to avoid the wall-slip effect. After the gap of the measuring system was set, the samples were left to rest for 5 min to allow material relaxation and to ensure thermal equilibrium.

2.4.3. Rotational shear (flow curves)

The experiments consisted of the following period intervals: first, a curve of ascending shear rate (upward flow curve) was logarithmically applied from 0.01 to 100 s^{-1}, and the response of shear stress was measured at 24 points; second, a constant shear rate of 100 s^{-1} was applied for 30 s, with 10 measurement points recorded; and, third, a descending shear rate curve (downward flow curve) was logarithmically applied from 100 to 0.1 s^{-1}, with 24 measurement points recorded. The Herschel-Bulkley flow model showed a good fit to both curves ($R^2 >$ 0.99) and was therefore chosen to model the flow behavior. In addition, the areas under the upward and downward flow curves were calculated using the trapezoidal sums, and the difference in area between the two curves was determined and presented as the hysteresis.

2.4.4. Oscillatory shear (viscoelastic behavior)

The linear viscoelastic character of all yogurts was determined through a strain amplitude sweep. The shear strain was varied logarithmically in the range from 0.01% to 300% at a constant angular frequency of 5 rad·s^{-1}, with 50 measurements recorded. The critical strain of the linear viscoelastic region, viscoelastic moduli values at this limit, and the yield point were calculated based on the elastic modulus versus strain or shear stress curves, and the flow index at the crossover point (G' = G″) was calculated.

2.5. Agavins and carbohydrate profiles in yogurt

2.5.1. Thin-layer chromatography (TLC)

Lyophilized yogurts samples were prepared at a final concentration of 100 mg·mL^{-1} in distilled water. Soluble carbohydrates were recovered in the supernatants by centrifugation at 3000 rpm for 10 min at room temperature. One microliter (1 μL) of supernatant (soluble carbohydrates) was applied to a silica gel TLC plate on an aluminum support. The TLC plate was developed in a mixture of butanol/propanol/water (3:12:4) as a solvent system, and carbohydrates were visualized by spraying a solution of aniline/diphenylamine/phosphoric acid in acetone and heating the TLC plate until spots were observed (Mellado-Mojica & López, 2015).

2.5.2. High-performance anion exchange chromatography coupled to pulse amperometric detection (HPAEC-PAD)

Yogurt samples were analyzed by HPAEC-PAD in accordance with the method established by Santiago-García et al. (2017) using a Dionex ion chromatograph system (ICS-3000; Dionex, USA), a CarboPac PA100 (4 × 50 mm) guard column, and a CarboPac PA100 (4 × 250 mm) analytical column (Dionex, USA). Lyophilized yogurt solutions were prepared at 0.5 mg·mL^{-1} final solution in deionized water (resistivity 28 mΩ). Solutions were filtered through a 0.45 μm nylon membrane before analysis, and 25 μL from each yogurt solution was injected into the column. Carbohydrate separation was performed on a sodium acetate gradient (0–500 mM) in NaOH solution (150 mM), with a column temperature of 25 °C and a flow rate of 0.8 mL·min^{-1}. The potentials applied for detection by the amperometric pulse were E1 (400 m s), E2 (20 m s), E3 (20 m s), and E4 (60 m s) of +0.1, −2.0, +0.6, and −0.1 V, respectively. Carbohydrate identification and concentrations were obtained by retention time comparison and analytical standards curves, respectively.

2.6. Fourier transform infrared (FTIR) spectroscopy of yogurt

Fourier-transformed mid infrared spectra were obtained using a Cary 660 FTIR spectrometer (Agilent Technologies, USA) equipped with an attenuated total reflectance (ATR) accessory and a diamond/Ge crystal plate (MIRacle by PIKE Technologies, USA). Lyophilized yogurts were used to minimize water interference during the spectral data collection. For measurement, 50 mg of lyophilized yogurt was placed onto the ATR crystal plate and 64 scans were recorded in the range of 4000–600 cm^{-1} at a nominal resolution of 4 cm^{-1} in transmittance mode (% T). Single-beam spectra of all samples were collected using air as the reference background. Three replicate measures of each sample were taken and the spectra were averaged. Spectral data were processed using the Resolutions-Pro FTIR spectroscopy software (Agilent Technologies, USA).

2.7. Chemometric analysis and spectroscopy

2.7.1. Principal component analysis

The processed spectral data from lyophilized yogurts were evaluated using discrimination analysis. Spectral data were exported to GRAMS spectroscopy software suite (Thermo Fisher Scientific, USA). Principal component analysis (PCA) was performed in the GRAM IQ software (Thermo Fisher Scientific, USA) to detect clustering in the data set and validated with the spectra of randomly selected samples that were not used to build the model.

Principal component models were developed for different regions of the FTIR: fingerprint (1800–800 cm^{-1}), fatty acids (3000–2700 and 1800–1700 cm^{-1}), carbohydrates (1200–800 cm^{-1}), and fructans (950–920 cm^{-1}) (Cozzolino, Roumeliotis, & Eglinton, 2014; Rodríguez-Saona & Allendorf, 2011; Santiago-García et al., 2017).

2.7.2. Loading factor components

The loading factor for each principal component (PC) was determined to infer the variables' influence in the model for the FTIR fingerprint region (1800–800 cm^{-1}) using GRAM IQ software (Thermo Fisher Scientific, USA) to determine how the contribution of the variable (wavenumber) influenced the classification and discrimination build models (Worley & Powers, 2013).

2.8. Sensory evaluation

The sensory characteristics of the yoghurt were evaluated by an untrained consumer panel of 42 persons at the University of Guanajuato (22 women and 20 men) who were frequent consumers of fermented milk drinks. Twenty milliliters of each of the six experimental yogurt variations were placed in plastic cups (4 °C ± 0.5 °C) 3 days after preparation, coded with three-digit numbers in a random manner, and presented randomly to the panelists. Consumers were asked to rate the appearance, odor, texture, flavor, and overall acceptability of yogurts using a 9-point hedonic scale (9 = like extremely, and 1 = dislike extremely) (Meilgaard, Civille, & Carr, 1999).

2.9. Statistical analysis

Statistical analyses were performed using Sigma Plot 11 (Sigma Plot 11 ©Systat Software). Values were expressed as mean values with their respective standard deviation. Statistical differences between groups were evaluated using one-way ANOVA followed by a minimum square difference or Tukey's test. A statistically significant difference between groups was considered to be present for P values of <0.05.

3. Results and discussion

3.1. Effect of addition of agavins on the physicochemical characteristics of yogurt

Yogurt samples containing agavins had a lower fat content than whole milk yogurt (1:4) (Table 2). The addition of different percentages of agavins from two *Agave* species to the low-fat yogurts compensated for the reduced fat content, resulting in an increase in viscosity of more than 5 times compared with the low-fat yogurts.

The pH values and acidity behavior for each yogurt sample at three different times (aged 1, 5, and 11 days) are presented in Fig. 1a. For all treatments, the pH was below 5; this was very important as the lower pH indicates an increase in the production of lactic acid, which allows reorganization and interconnection of the casein and the resulting increase in texture (De Vuyst et al., 2001; Duboc & Mollet, 2001; Laws et al., 2001; Purwandari, Shah, & Vasiljevic, 2007). In other words, the pH and the titratable acidity were not influenced by the addition of the agavins: the acidity ranged from 75°D to 87°D and remained practically constant ($p > 0.05$) after 11 days in refrigerated conditions. Guven, Yasar, Karaca, and Hayaloglu (2005) found similar results during the aging of low-fat yogurt containing inulin.

The total solid content of all yogurts ranged between 16.2 and 20.03 g/100 g of yogurt. The added agavins led to an expected increase on the concentrations of total solids in yogurts (Table 2).

Whey separation (wheying-off or syneresis) is defined as the expulsion of whey from the network, which then becomes visible as surface whey. Wheying-off negatively affects consumer acceptance of products as consumers infer there is a microbiological issue with the product (Lee & Lucey, 2010). The physicochemical properties of the yogurts were determined at the beginning and during the storage period in refrigeration. The addition of agavins affected the syneresis and water retention capacity values: a significant difference was observed ($P < 0.05$) in both values. The values of the water retention capacity were higher than those found in syneresis; which may have been due to the centrifugal force that breaks the structure between fructan-protein-fat, causing greater release of water. Yogurts supplemented with agavins retained a higher quantity of serum compared with low-fat yogurt (YFRC), as shown by the decreased syneresis and greater water retention capacity values. From the results presented in Table 2, it can be seen that at higher agavin concentration, a greater reduction in syneresis occurs. For example, yogurts with 6% agavins (YFR_AA6%, YFR_AP6%) presented the lowest values, but they were not significantly different from YFR_AA3%; however, YFR_AP3% had greater firmness and creaminess values. According to Folkenberg, Dejmek, Skrive, and Ipsen (2006), agavins have a great capacity to retain serum, resulting in low levels of syneresis and causing a similar to effect as exopolysaccharides. In the same way, Guggisberg, Cuthbert-Steven, Piccinali, Bütikofer, and Eberhard (2009) showed that the addition of 2% inulin resulted in greater firmness and creaminess, reducing the syneresis in yogurt made with low-fat milk.

The reduction of syneresis by agavin supplementation can be explained by their highly branched structure, which can easily interact with other components and form bonds with the protein matrix, ions in the aqueous phase, and water. Espinoza-Andrew & Rodríguez-Rodríguez (2018) reported that agave fructans have a greater affinity for water than inulin, forming a gel-like network within the yogurt matrix and consequently affecting the viscosity. Futhermore, agavin molecules can act as part of the structural protein network by forming complex casein aggregates, as reported by Crispín-Isidro, Lobato-Calleros, Espinosa-Andrews, Alvarez-Ramírez, and Vernon-Carter (2015), which provides stability to the protein network, most likely because the OH^- ions of polysaccharides interact through hydrogen bonds with charged residues on the surface of the protein, further controlling syneresis.

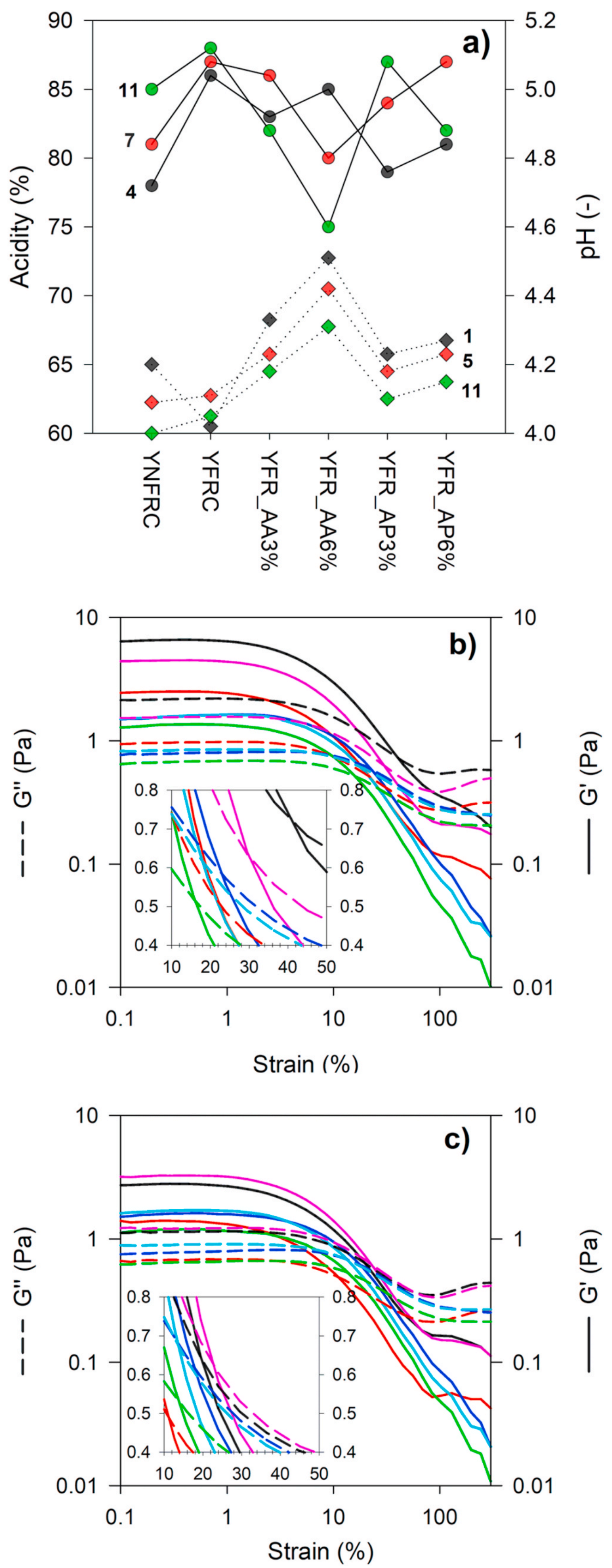
Table 2
Physicochemical properties of yogurt samples. Sample nomenclature is listed in Table 1.

Sample	Fat (%)	Total solids (%)	Acidity (°D)	Viscosity (cP)	Syneresis (%/w)	WRC (g/100 g)
YNFRC	4.0 ± 0.08^{b}	17.75 ± 0.45^{a}	72.70 ± 0.52^{b}	1360 ± 58.24^{d}	0.15 ± 0.07^{a}	0.870 ± 0.06^{a}
YFRC	0.8 ± 0.10^{a}	16.05 ± 1.28^{a}	72.33 ± 0.41^{b}	89 ± 17.30^{a}	3.01 ± 0.82^{b}	3.6150 ± 0.01^{c}
YFR_AA3%	0.8 ± 0.10^{a}	17.90 ± 1.79^{b}	71.08 ± 1.23^{b}	415 ± 40.29^{c}	0.85 ± 0.14^{a}	2.686 ± 0.01^{bc}
YFR_AA6%	1.0 ± 0.12^{a}	20.07 ± 2.03^{c}	66.09 ± 3.45^{a}	468 ± 28.91^{c}	0.75 ± 0.12^{a}	2.300 ± 0.01^{b}
YFR_AP3%	0.9 ± 0.09^{a}	18.37 ± 2.16^{b}	72.93 ± 1.26^{b}	385 ± 42.30^{b}	0.66 ± 0.13^{a}	2.799 ± 0.00^{bc}
YFR_AP6%	1.0 ± 0.09^{a}	19.49 ± 2.49^{b}	68.00 ± 3.42^{a}	418 ± 31.830	0.55 ± 0.11^{a}	2.310 ± 0.02^{b}

°D, °Domic (1°D = 0.1 g of lactic acid/L).
[a]Syneresis expressed in weight percent; WRC, Water retention capacity. Values represent mean ± standard deviation (SD). Means sharing the same letter do not differ significantly ($P \leq 0.05$).

3.2. Rheological properties

The Brookfield viscosity measurements of the yoghurts prepared with different treatments are shown in Table 2. The percentage of agavins influenced the viscosity of the yogurts. A direct relationship was observed between viscosity and agavin concentration; a similar relationship was previously reported with inulin, a linear polysaccharide (Guggisberg et al., 2009). All reduced-fat yogurts containing agavins from *A. angustifolia* (YFR_AA3% and YFR_AA6%) and *A. potatorum* (YFR_AP3% and YFR_AP6%) had higher viscosity values (385–468 cP) than YFRC yogurts, which had the lowest viscosity (86 cP). The addition of agavins to reduced-fat yogurts can support the formation of microcrystals when sheared in milk. These crystals are not discretely perceptible, but interact to form a smooth creamy texture. Agavins can also contribute to gel strength. Therefore, agavins contribute to a pleasant sensation in the mouth. Moreover, they did not contribute to flavor, but increased viscosity and hardness. The viscoelastic moduli versus shear-strain amplitude for yogurt samples containing agavins is



(caption on next column)

Fig. 1. (a) Acidity level (circle) and pH (rhombus) of yogurt samples after different aging periods and storage at 4 °C; (b) viscoelastic moduli (G′ and G″) versus the shear strain amplitude after 1 day of storage; and (c) viscoelastic moduli (G′ and G″) versus the shear strain amplitude after 11 days of storage; the inset plots in (b) and (c) show the region of crossover points on a linear scale. YNFRC, black; YFRC, red; YFR_AA3%, green; YFR_AA6%, pink; YFR_AP3%, dark blue; YFR_AP6%, light blue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

shown in Fig. 1b and c.

The results of 1-day aged and 11-day aged samples are shown in Fig. 1b and c, respectively. All yogurts exhibited characteristics typical of a weak viscoelastic gel (G'>G″) and strain-thinning behavior. After 1 day of aging, yogurts containing agavins had lower G′ and G″ values than the corresponding yogurt made using whole milk (YNFRC), but after 11 days of aging, the yogurts with 6% agavins from *A. angustifolia* had higher G′ and G″ values than YNFRC. YFRC exhibited lower viscoelastic moduli values compared with whole milk yogurt. The age of yogurt significantly reduced the crossover point (G' = G″). The linear strain for the linear viscoelastic region was between 1.11% and 2.78%, which was in the range reported for yogurt (Crispín-Isidro et al., 2015; Pang, Deeth, Prakash, & Bansal, 2016; Staffolo, Bertola, Martino, & Bevilacqua, 2004).

The yield point and flow index are presented in Table 3. The incorporation of agavins increased these values compared with a pure skimmed milk yogurt, with higher values at both time points for the formulations containing agavins. However, despite using the highest concentration of both agavins, the yield point and flow index were almost half the values for the yogurt prepared with whole milk, indicating that the protein network in yogurts containing fructans was weaker than that in whole milk yogurt. This may be attributable to the reduction in crosslinking between casein micelles in the gel network, which may be caused by the presence of fructan molecules between casein micelles, reducing the crosslinking sites. Crispín-Isidro et al. (2015) showed that fructans from *Agave tequilana* covered the casein micelles and produced less dense, more porous, and less fused casein micelle aggregates than whole milk yogurt.

The flow curves fitted well to the Herschel-Bulkley model; the results are summarized in Table 3. The Herschel-Bulkley yield stress and consistency index were reduced with aging, and they were almost two decimals lower than formulations found in the literature (Pang et al., 2016; Sah, Vasiljevic, McKechnie, & Donkor, 2016; Staffolo, Dello, Bertola, Martino, & Bevilacqua, 2004). All the prepared formulations had the rheological properties of drinkable yogurts. Shear-thinning behavior was observed in the shear rate range from 0.01 to 10 s^{-1}; above 10 s^{-1}, shear-thickening behavior was observed ($n > 1$). Flow behavior index "n" values were lower for the downward than the upward flow curves. Yogurts prepared with skimmed milk showed higher values of "n" in comparison with whole milk yogurt, indicating that the fat was the main factor affecting the flow index. One possible explanation is the fat globules trapped in the yogurt network enhanced the flowability of the yogurt.

The addition of both agavins to low-fat yogurt compensated for the lower number of milk-fat globules with saccharide chains, with fructans from *A. potatorum* more effective in extending the linear viscoelastic region. The values of consistency index ranged from 0.005 to 0.067 Pa·s^n, with lower values for skimmed milk yogurts than whole milk yogurt, suggesting the low-fat yoghurt had a less viscous nature. However, skimmed milk yogurts containing fructans had higher consistency index values than YFRC. Therefore, the addition of agavins to skimmed milk yogurt increased the number of stress-carrying interactions and the strength of the gels.

Table 3
Rheological properties of yogurt samples at 1 and 11 days after preparation and storage under refrigeration (4°–10 °C). Sample nomenclature is listed in Table 1.

Sample[d]	Yield stress[a]	K[a]	n[a]	LVE region[c]	G'[b]	G''[b]	Yield stress[b]	Flow stress[b]
	(Pa)	(Pa·s^n)	(−)	(%)	(Pa)	(Pa)	(Pa)	(Pa)
YNFRC[1]	0.711	0.075	1.191	1.515	6.176	0.806	0.113	0.415
	0.566	0.035	1.104[c]					
YFRC[1]	0.209	0.010	1.502	1.468	1.212	0.638	0.027	0.090
	0.184	0.005	1.368					
YFR_AA3%[1]	0.197	0.015	1.343	1.949	1.251	0.692	0.033	0.116
	0.167	0.009	1.195					
YFR_AA6%[1]	0.491	0.067	1.257	1.363	3.067	1.566	0.075	0.267
	0.435	0.024	1.221					
YFR_AP3%[1]	0.300	0.015	1.392	2.783	1.582	0.819	0.061	0.188
	0.247	0.006	1.339					
YFR_AP6%[1]	0.245	0.021	1.426	2.477	1.578	0.852	0.053	0.155
	0.239	0.005	1.457					
YNFRC[2]	0.377	0.053	1.151	1.341	2.587	1.147	0.044	0.180
	0.274	0.060	0.893					
YFRC[2]	0.221	0.016	1.452	1.119	1.306	0.688	0.021	0.077
	0.189	0.007	1.335					
YFR_AA3%[2]	0.206	0.013	1.324	2.190	1.118	0.665	0.032	0.104
	0.156	0.014	1.070					
YFR_AA6%[2]	0.388	0.053	1.180	1.489	3.029	1.237	0.055	0.199
	0.339	0.037	1.109					
YFR_AP3%[2]	0.282	0.015	1.335	2.295	1.503	0.810	0.047	0.154
	0.219	0.011	1.152					
YFR_AP6%[2]	0.249	0.020	1.350	1.839	1.617	0.905	0.039	0.131
	0.214	0.011	1.240					

[a] Corresponding to fitting of flow curves to the Herschel-Bulkley model ($0.993 \leq R^2 \leq 0.999$).
[b] Corresponding to those values computed from the oscillatory shear-strain amplitude sweep.
[c] Data in bold correspond to the downward flow curve fittings.
[d] Samples storage at 4 °C (1 and 11 storage days, 1 and 2 superscripts respectively).

3.3. *Carbohydrates profile and composition in yogurt samples*

Carbohydrate profile analysis is a valuable tool on the food products analysis, therefore, TLC and HPAEC-PAD were carried out to evaluate the final products (yogurts) composition, as well as, to determinate the agavins integrity.

3.3.1. *Thin-layer chromatography*

Using TLC, we analyzed yogurt non-fat and fat reduced control samples, LDP-agavins extracted from *A. angustifolia* and *A. potatorum* species, as well as yogurt fat reduced samples added with agavins (3 and 6%) from different agave species along with standard mixtures of fructooligosaccharides (F) and maltooligosaccharides (M) (Supplementary material Fig. 1S). A typical agavins profile, polydisperse mixture of oligo- and polysaccharides were observed for the LDP-agavins extracted fructans (Mellado-Mojica & López, 2012) with a predominance of long chain carbohydrates. In all yogurt samples (non-fat and fat reduced) a bluish spot identified as lactose was the most abundant carbohydrate, followed by sucrose and glucose traces. Yogurt added samples, besides lactose and sucrose, also, show the agavins presence in the final products. The above are in accordance with the previously describe by Crispín-Isidro et al. (2015) were they suggested that inulin and agavins from *Agave tequilana* remain intact in final product. In fact, this is the first work were agavins presence is demonstrate in dairy products.

3.3.2. *HPAEC-PAD profiles*

HPAE-PAD chromatography is a powerful, sensitive, and reliable analytical tool for the qualitative (profile) and quantitative analysis of oligosaccharides and polysaccharides with different chain lengths. Agavins are very complex mixture of oligo and polysaccharides when compared to linear fructans such inulin. HPAEC-PAD analyses were carried out to characterize the carbohydrates profiles from LDP-agavins (*A. angustifolia* and *A. potaturum* species), as well as, the yogurt samples.

A typical agave fructans profiles were observed in agavins employed as fat replacers, were a great diversity of isomers (peaks) with the same degree of polymerization could by observed (Supplementary material Fig. 2S), no significant differences were determined when fructans from *A. angustifolia* (Supplementary material Fig. 2S(a) and *A. potatorum* (Supplementary material Fig. 2S(b) were compared. Whilst no oligo- or polysaccharides were observed in the yogurt non-fat and fat reduced controls, Supplementary material Fig. 2S(c) and (d), respectively).

TLC and HPAEC-PAD analyses confirmed that lactose and sucrose were the most abundant disaccharides found in the yogurt samples, both controls (non-fat and fat reduced) and agavins added (Supplementary material Fig. 3S). Fig. 2 shows the HPAEC-PAD profiles of all yogurts supplemented with agavins, exhibiting quantitatives but not qualitatives differences, because there are no differential peaks among treatments, but the identified peaks presented differences in their abundances; this is consistent with the percentage of fructans added to each treatment (3 and 6%). Compiled information are in agreement with data observed by TLC were fructans differences were showed according their fructans amount in the sample.

In addition, HPAEC-PAD profiles demonstrate that the process of yogurt elaboration under the proposed protocol did not alter the composition of fructans, ensuring that they arrive intact to the consumer. The above is in accordance with Guven et al. (2005) and Aryana et al. (2007) were they report the fructans inulin type in fat-free yogurt, as well as, with the Santiago-García et al. (2017) findings, where they demonstrate by HPAEC-PAD that agavins remain intact along the bakery manufacture. Due to the above, agavins in dairy products become as a good prebiotic dietary fiber in low-fat products and that might elicit some health benefits.

3.4. *FTIR spectroscopy analysis*

Vibrational spectroscopic techniques such mid infrared (FTIR) spectroscopy combined with chemometrics analysis have been used as a non-destructive and easy way to quantify and characterize different biological samples. These techniques can quickly provide a large amount of information on any type of sample, therefore have been used extensively to determine components such as carbohydrates, fats, amino acids, proteins, and moisture, among many others in food products

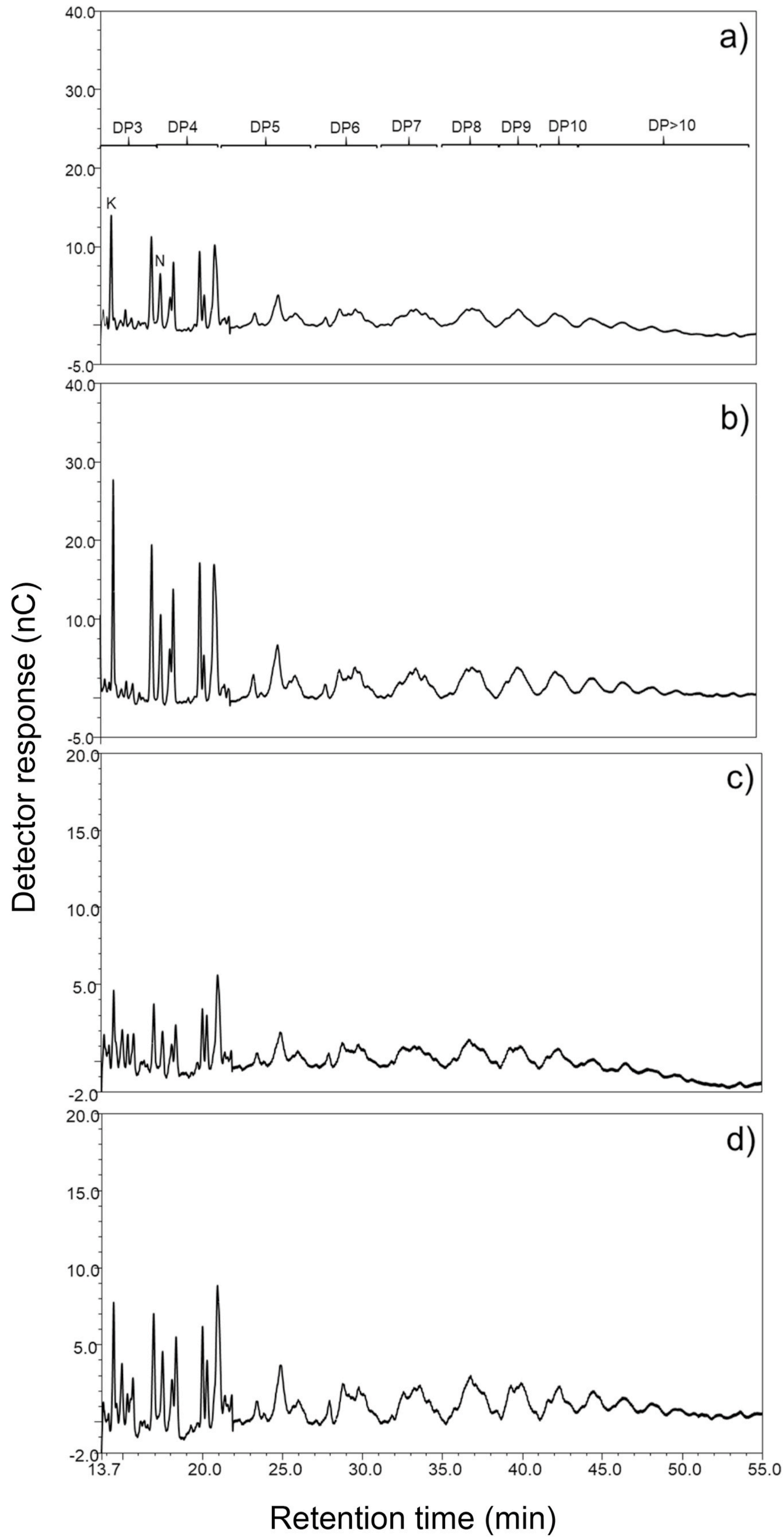


Fig. 2. HPAEC-PAD profiles of carbohydrate composition yogurt samples. a) Yogurt fat reduced added with *A. angustifolia* agavins 3% (YFR_AA3%), b) yogurt fat reduced added with *A. angustifolia* agavins 6% (YFR_AA6%), c) yogurt fat reduced added with *A. potatorum* agavins 3% (YFR_AP3%) and d) yogurt fat reduced added with *A. potatorum* agavins 6% (YFR_AP6%). Samples nomenclature is listed in Table 1.

(Rodríguez-Saona & Allendorf, 2011).

FTIR analysis were carried out for yogurts samples, both controls (non-fat and fat reduced) and fat-reduced agavins added yogurts (see Fig. 3). The yogurt samples FTIR spectra over the region 600-4000 cm^{-1} exhibited strong absorption bands in the carbohydrates (900-1200 cm^{-1}) and fatty acids (1700-1800 cm^{-1} and 2700-3000 cm^{-1}) regions. Fingerprint region (800-1800 cm^{-1}) was determined as the region with mayor chemical groups contribution, dominate by the absorption bands derived from the glycosidic linkages bending and stretching modes (*C*–O and *C*–H) from the carbohydrates and fructans compound in samples (Wang, Kliks, Jun, Jackson, & Li, 2010; Cozzolino et al., 2014; Mellado-Mojica & López, 2015), and carboxylic (C═O) group stretching band at 1700-1800 cm-1) from the fatty acids (Guillen and Cabo, 1997; Rodríguez-Saona & Allendorf, 2011). In addition, stretching vibrations for the hydrocarbon chain (*C*–H, CH_2) exhibited strong absorption at 2700-300 cm^{-1} (Guillen and Cabo, 1997).

Differences among samples were observed according their fat and fructans contents. Yogurt non-fat reduced control sample showed strong signals in the fatty acids region, whereas that yogurt fat reduced control and agavins added samples exhibited similar intensities in the above region. No significant differences were identified in the carbohydrates region among samples, however, those FTIR spectra are not easy to understand or interpret. Therefore, application of chemometrics tools for characterization, determination of chemical compounds and quality control of food products has recently become a very active research area, which will be very useful in the yogurt samples analysis.

3.5. Principal Components Analysis (PCA) of yogurt

Principal Components Analysis (PCA) gave an overview of the similarities and differences among samples and the relationships between their chemical components. In addition, PCA models often provide a relatively simple representation of similarities between samples based on more-or-less complex analytical data.

PCA models form the FTIR analyzed regions 800-1800 cm^{-1} (fingerprint); 1700–1800 and 2700-3000 cm^{-1} (fatty acids); 800-1200 cm^{-1} (carbohydrates) and 920-950 cm^{-1} (fructans) are shown in Fig. 4.

PCA model from the FTIR spectra from the fingerprint coupled to fatty acids region showed that non-fat reduced yogurts (YNFRC) were classified away from the fat-reduced samples group (Fig. 4a). Yogurt fat reduced control and agavins added samples were separated into two groups, one for the yogurt fat reduced added with agavins from *A. angustolia* (YFR_AA3% and YFR_AA6%); and a second group fat reduced control samples (YFRC) was grouped with the yogurt fat reduced added with agavins from *A. potatorum* treatments (YFR_AP3% and YFR_AP6%). PCA models from the two fatty acids regions (1700-1800 cm^{-1} and 2700-3000 cm^{-1}) exhibited similar classifications for the fingerprint/fatty acids PCA model (Fig. 4b). On the other hand, PCA models results using the general carbohydrate region displayed a good separation by quadrants, where agavins addition to the sample were separated according to the agavins amount and agave species; whereas that the cluster YNFRC was grouped closed to YFRC, because these samples were not added with agavins. Finally, PCA for the analysis of the fructans region displayed identical behavior than carbohydrates PCA models (Fig. 4d); when a yogurt fat reduced samples added with agavins from a different Agave species or similar amount are closer together, than those without agavins supplementation.

Based on the above, it is clear that carbohydrates and fatty acids have a strong contribution on the samples classification. Data results are in accordance with the reported by Santiago-García et al. (2017) where a sample classification according to their fat content and fructans addition was observed in cookies.

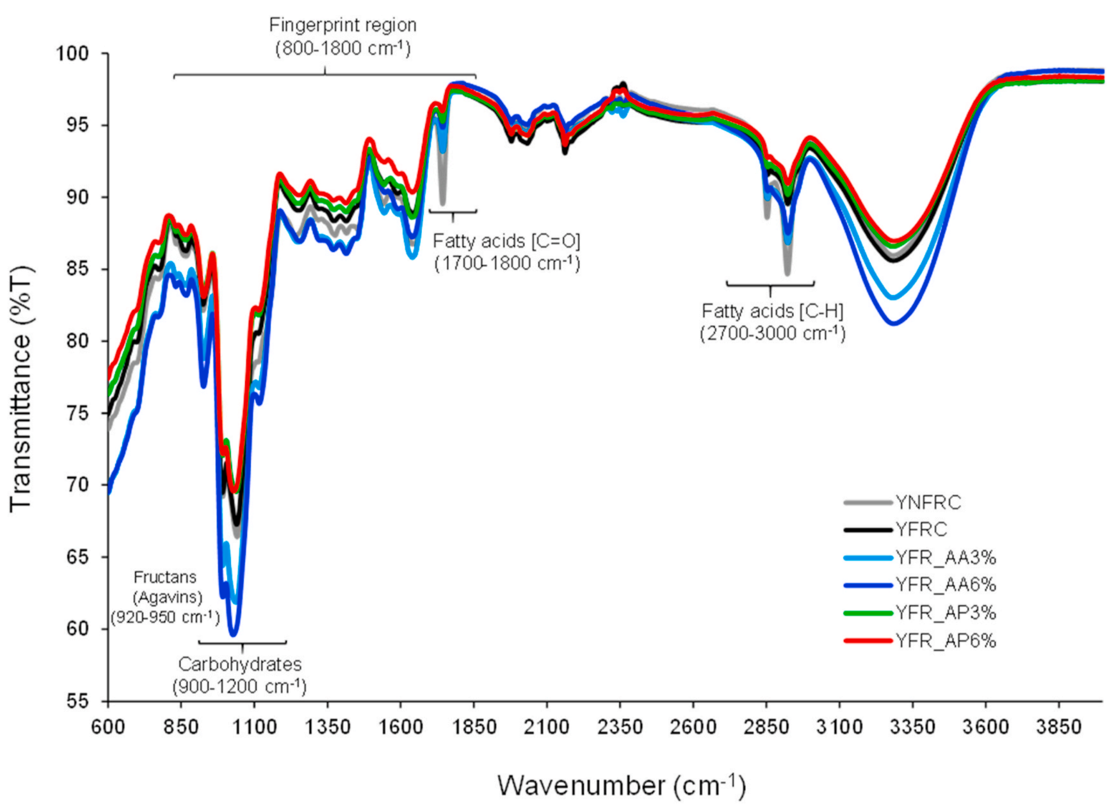


Fig. 3. FTIR spectra from yogurt lyophilized samples: controls (non-fat & fat reduced) and fat reduced yogurt added with agavins (LDP) from *Agave angustifolia* and *A. potatorum* species. YNFRC, Yogurt non-fat reduced control (gray color); YFRC, yogurt fat reduced control (black color); YFR_AA3%, yogurt fat reduced added with *A. angustifolia* agavins 3% (light blue color); YFR_AA6, yogurt fat reduced added with *A. angustifolia* agavins 6% (dark blue color); YFR_AP3%, yogurt fat reduced added with *A. potatorum* agavins 3% (green color); YFR_AP6%, yogurt fat reduced added with *A. potatorum* agavins 6% (red color). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

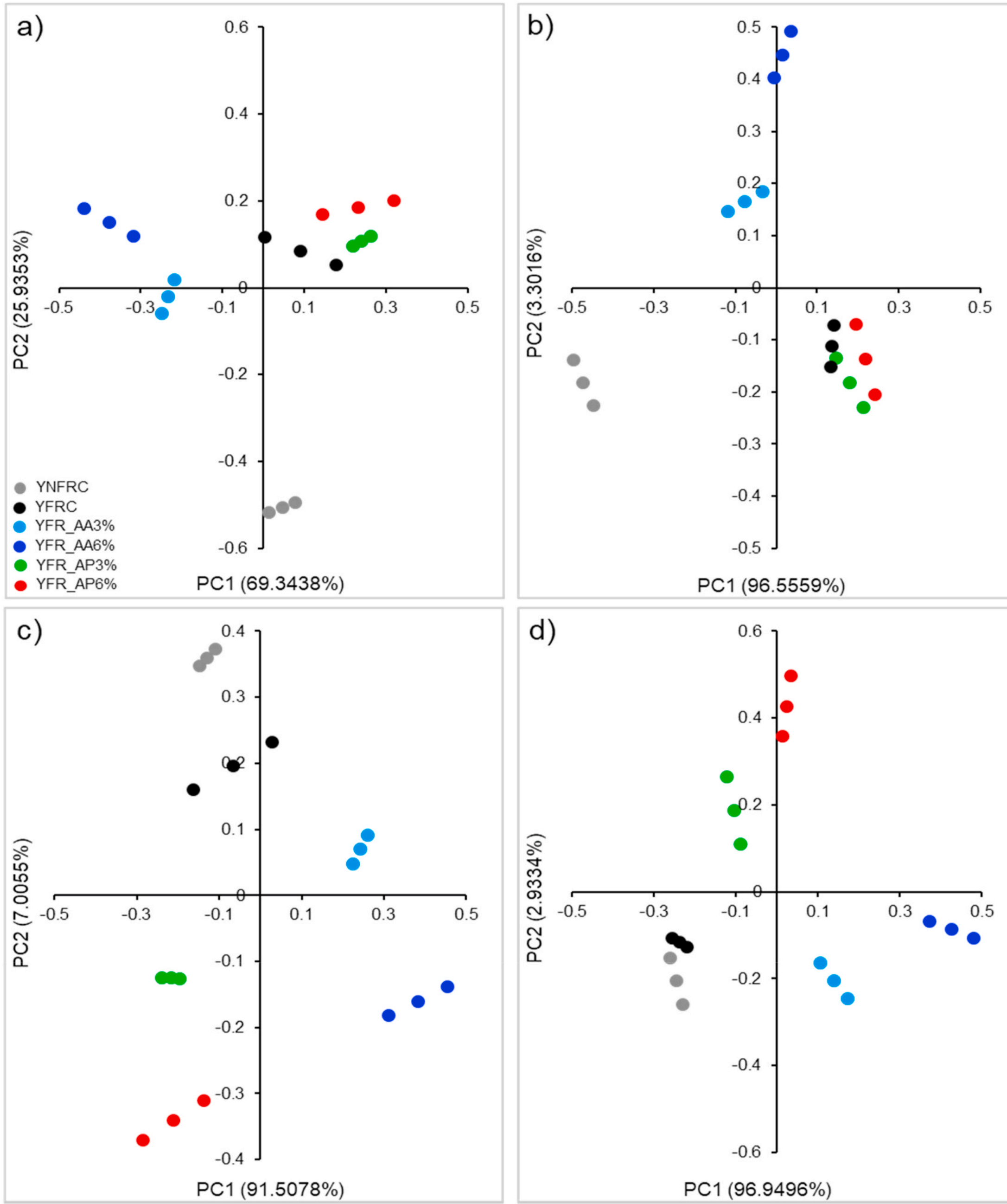


Fig. 4. Principal component analysis (PCA) models from the FTIR spectra regions: a) Fingerprint (800-1800 cm-1) and fatty acids (2700-3000 cm-1); b) fatty acids (1700–1800 and 2700-3000 cm-1); c) carbohydrates (800-1200 cm-1); d) fructans (920-950 cm-1). YNFRC, yogurt non-fat reduced control (gray color); YFRC, yogurt fat reduced control (black color); YFR_AA3%, yogurt fat reduced added with *A. angustifolia* agavins 3% (light blue color); YFR_AA6, yogurt fat reduced added with *A. angustifolia* agavins 6% (dark blue color); YFR_AP3%, yogurt fat reduced added with *A. potatorum* agavins 3% (green color); YFR_AP6%, yogurt fat reduced added with *A. potaturorum* agavins 6% (red color). Samples nomenclature is listed in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In order to identified which component influence more the PCA models, as well as to understand their samples classification, the factor loading component from the PCA model using the FTIR spectra from the fingerprint region coupled to fatty acids region (Fig. 4a) was built and graphed (Supplementary material Fig. 4S). Carbohydrates region (900-1200 cm^{-1}) showed a stronger contribution on the PC1 (69.34%) whilst fatty acid region from the carboxylic groups (1700-1800 cm^{-1}) mainly dominate the PC2 (25.93%), therefore samples classification was carried out based on the amount of these compounds.

Finally, FTIR-PCA can be used in the food industry as a rapid, inexpensive, and simple alternative to determine both the sample chemical composition, and their classification/discrimination according their treatments.

3.6. Sensory evaluation

The sensory attributes related to the texture of yogurts are presented in Fig. 5. Global acceptability is a complex and multidimensional term comprising the combination of different dynamics and variable sensory perceptions. The general acceptability scores for YFR_AA3%, YFR_AP3%, and YFR_AP6% were not significantly different (P $\geq$ 0.05) compared with formulation YNFRC (6.88), and had slightly higher

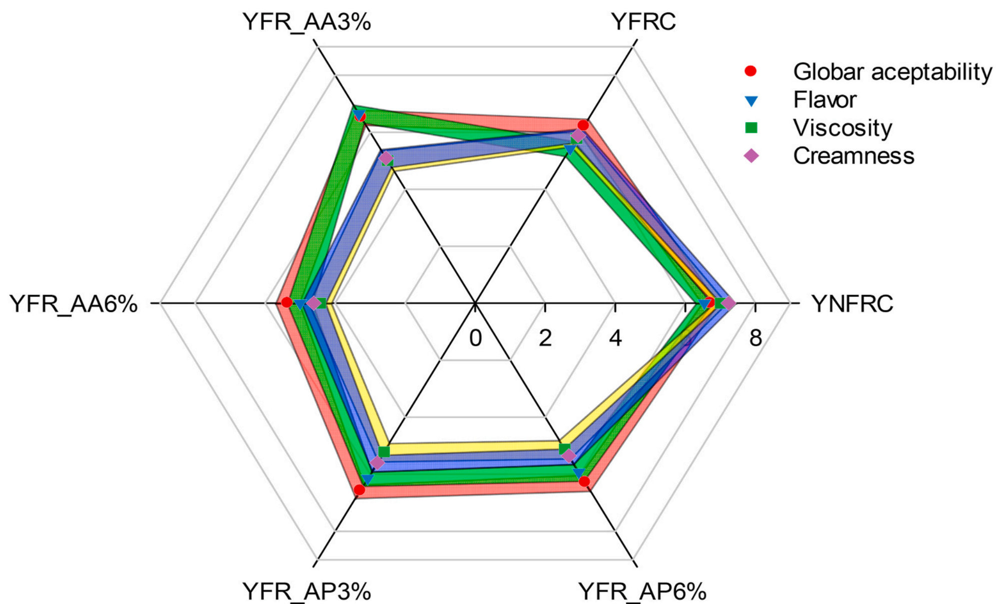


Fig. 5. Sensory attributes from yogurt samples. Sample nomenclature is listed in Table 1.

values for RG (6.21), conferring a similar level of global acceptability as full-fat yogurt. In this respect, the level of addition of YFR_AA3% or YFR_AP3%, or YFR_AP6% resulted in a different impact on the sensory attributes evaluated.

With regard to taste, the formulations YFR_AA3% (6.64) and YFR_AP3% (6.14) had highly similar values to YNFRC. The sensation of viscosity experience with YFR_AA3% (5.0), YFR_AP3% (5.21), and YFR_AP6% (5.11) was preferred over that of YFR_AA6% (4.42). No significant differences were found in the sensation of the viscosity of the samples supplemented with agavins. This may indicate that the sensory analysis was not sensitive enough to highlight the differences that were previously observed instrumentally. Dello, Bertola, Martino, and Bevilac-Qua (2004) found no differences in viscosity and acceptability between low-fat yogurt, yogurt with 1.3% inulin added, and a yogurt control with full fat. In terms of creaminess, the scores were slightly lower than for the low-fat yogurt. The incorporation of agavins from *A. potatorum* at higher levels (6%) significantly improved the perceived creaminess of the product ($p < 0.05$); similarly, in this formulation, the mouth feel of the products also improved, compared with the low-fat formulation, and the resulting texture was perceived as softer. Kip, Meyer, and Jellema (2006) reported that 3% inulin can be used to improve mouth feel of low-fat yogurt.

Sensory attributes such as color, odor, surface appearance, and taste were not affected by the incorporation of agavins into any of the formulations. The panelists tested a conventional full-fat yogurt as a matrix to rate the sensory attributes; as the test was blinded, no information on the composition of yogurts was provided to the panelists.

4. Conclusion

The addition of agavins from *A. angustifolia* and *A. potatorum* modified the sensory and rheological properties of fat-reduced yogurt. Yogurts containing the agavins showed lower levels of syneresis and five-fold greater viscosity compared with low-fat yogurt. Rheological and physicochemical properties were dependent on the agavin concentration and the species of agave. The addition of agavins to the low-fat yogurt compensated for the lower number of milk-fat globules with saccharide chains, and the agavins from *A. potatorum* were more effective in extending the linear viscoelastic region. HPAEC-PAD analysis revealed the presence of HDP agavins in the final product. Therefore, agavins can be used in low-fat yogurt formulations to obtain acceptable properties compared with whole milk yogurt and better properties than skimmed-milk yogurt, to function as texturizers, to improve the viscosity of the final product, and to stabilize the final product through interactions with water, and contributing to a decrease in syneresis. In addition, they are a good source of prebiotics that act synergistically with the nutritional and therapeutic properties of yogurt to produce health-beneficial effects. Infrared spectroscopy allowed the identification, classification, and discrimination of different food materials and, in our study, was used to support the differences each of the treatment via PCA.

CRediT authorship contribution statement

Patricia A. Santiago-García: Conceptualization, Methodology, Writing - review & editing, Writing - original draft, Data retention, writing, and preparation of the original draft, Writing - review & editing, Writing, reviewing, and editing. **Erika Mellado-Mojica:** Conceptualization, Methodology, Writing - review & editing, Writing - original draft, Data retention, writing, and preparation of the original draft, Writing - review & editing, Writing, reviewing, and editing. **Frank M. León-Martínez:** Writing - review & editing, Writing - original draft, Data retention, writing, and preparation of the original draft, Writing - review & editing, Writing, reviewing, and editing. **Jorge G. Dzul-Cauich:** Contributed to the discussion; . **Mercedes G. López:** Head of the group. **M. Isabel García-Vieyra:** Conceptualization, Methodology, Writing - review & editing, Writing - original draft, Data retention, writing, and preparation of the original draft, Writing - review & editing, Writing, reviewing, and editing.

Declaration of competing interest

The authors declare that they do not have any conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110846.

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