Relationship between carbohydrate composition and fungal deterioration of functional strawberry juices preserved using non-thermal treatments

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ABSTRACT

BACKGROUND: The quantification of the main carbohydrates present in strawberry juices enriched with inulin and fructo-oligosaccharides (FOS) and preserved by non-thermal techniques (vanillin and ultrasound) were studied, as well as the evolution of these compounds and their relationship with fungal deterioration during 14 days of refrigerated storage.

RESULTS: A simple and environmentally friendly analytical approach based on high performance liquid chromatography with reflection index detector was developed for simultaneous determination of inulin, FOS and mono and disaccharides present in the juices. When analyzing the evolution of carbohydrates during storage, a direct relationship between the consumption of sucrose and the growth of yeasts and molds (main spoilage flora in strawberry) was observed, especially in untreated samples (control). On the contrary, no sucrose consumption was observed during storage of treated sample, thus demonstrating the efficiency of the non-thermal treatments to control yeasts and molds growth. In turn, inulin and FOS added to juices were not degraded during storage.

CONCLUSION: The results demonstrated that non-thermal treatments are adequate to prevent the growth of deteriorative flora in strawberry juices and adding inulin and FOS can be a good strategy to functionalize them, improving their nutritional properties.

Keywords: carbohydrates, non-thermal techniques, yeasts and molds, fruit-based product, functional ingredients.

INTRODUCTION

The consumption of fruits in the form of juices is a common habit in the nowadays population worldwide, with few time for eat and an increasing trend to consume ready-to-eat products. For this reason, the production of juices, involving fruits manipulation and storage, requires adequate treatments to ensure their safety. In line with the consumer demand of "fresh-like" products, the juice industry is getting committed in the development of innovative and safe fresh fruit beverages containing high concentrations of functional ingredients, [1] and in the replace of conventional thermal treatments with non-thermal ones, and synthetic additives with natural products. [1, 2] A functional food or functional ingredient is considered to be any food or food component that provides health benefits beyond basic nutrition. [3] The incorporation of inulin and fructo-oligosaccharides (FOS), two ingredients with well-established capacity to modulate the composition of the intestinal microbiota (prebiotics), [4] has recently demonstrated to be a good strategy to improve the nutritional value of strawberry juices. [5] Fructans are polymers of fructose consisting in fructose units linked by (2→1)-β-glycosidic bonds and a single D-glucosyl unit at the non-reducing end. They differ in their degree of polymerization (DP), the presence of branches, the type of linkage between adjacent fructose units, and the position of the glucose residue. Fructans with DP from 2 to 10 are commonly known as FOS, whereas larger fructans (DP from 10 to 60 or more) are generally referred as inulin. [6]

Strawberry juices are mainly composed of sugars (sucrose, glucose and fructose) and low concentrations of organic acids (mainly citric acid). [7] As sugars are excellent sources of carbon and energy for the growth of deteriorative microorganisms, the storage of juices

requires adequate treatments. Non-thermal processing techniques have the potential to offset some of the unwanted reactions in foods resulting in undesirable organoleptic, texture and nutritional effects, often associated with thermally processed foods. [1] Among these treatments, ultrasound (sonication) is a common and environmentally friendly process to destroy deteriorative microorganisms and enzymes. [8, 9] Its efficiency can be enhanced when combined with naturally occurring antimicrobials, such as vanillin (4-hydroxy-3-methoxybenzaldehyde). [10]

The knowledge about the quantitative composition of sugars in fruits based products is of paramount importance since these compounds are considered as significant quality factors by both consumers and the food industry. [11] Taking into account that juice spoilage associated with microbial metabolism leads to sugar consumption, monitoring the sugar composition of juices (including inulin and FOS in the cases they are added as functional ingredients) during storage could be a good indicator of the efficiency of preservation treatments.

High Performance Liquid Chromatography (HPLC) has been used for fructans and carbohydrate separation and quantification. [12, 13] To this aim, different HPLC detectors, including refractive index (RI), evaporative light scattering (ELSD), and electrochemical (ECD) detectors are commonly used. They differ in their sensitivity and compatibility with the use of gradients. [14] Therefore, obtaining satisfactory results depends on optimizing the associated mobile phase composition and detectors, as well as on preparing the samples for HPLC. [15]

Although the composition of fructose, glucose and sucrose in fruit-based products has been widely studied, [7, 16] their relation with spoilage flora growth of strawberry juice has not been addressed.

In our previous work, [10] the evolution of quality parameters (microbiological, nutritional and sensory) of strawberry juice enriched with inulin and FOS and treated with non-thermal processing (vanillin combined with ultrasound) was studied during 14 d of storage at 5 °C. These results proved that preservation treatments applied, improve almost every quality attribute during storage and reduce microbial growth. However, some issues remain unknown, such as the effect of the application of these non-thermal techniques on the stability of the added functional ingredients during storage or their performance in the typical acidic environment of strawberry juices. In addition, to the best of our knowledge, the quantification of inulin and FOS added to functionalize food products (such as strawberry juice) has not been previously investigated. Therefore, the aims of this work were: (1) to develop a method based on HPLC with RI detector to separate and quantify the main carbohydrates present in fiber-enriched strawberry juices (namely fructose, glucose, sucrose, FOS and inulin) preserved by non-thermal processing during storage; (2) to establish a relationship between the consumption of sugars and the yeasts and molds inactivation; (3) to investigate the stability of exogenously added inulin and FOS during storage.

EXPERIMENTAL

Materials

Inulin (purity: 92%, DP average: 25) and FOS (purity: 95%, DP average: 4) were obtained from Saporiti (Buenos Aires, Argentina). Vanillin was purchased from Firmenich SAICYF (Argentina). Sucrose, glucose, fructose, inulin, high-metoxyl pectin and citric acid were obtained from Sigma Chemical (St. Louis, MO, USA). 1-kestose (DP3), nystose (DP4)

and 1^F-fructofuranosyl nystose (DP5) standards were purchased from Wako Chemicals (Richmond, VA, USA).

Juice obtaining

Strawberries (*Fragaria x ananassa* Duch.) were grown and harvested in Sierra de los Padres (Mar del Plata, Argentina). After discarding defective fruits, those of good quality were washed with tap water and the calyx was hand removed. Strawberry juice was prepared by squeezing the fruits with a commercial juice extractor (Moulinex, Buenos Aires, Argentina). The obtained strawberry juice was collected in a glass jar. The juice was homogenized and divided in three portions, which were bottled into 100 mL polyethylene terephthalate flasks under hygienic conditions and sealed with polyethylene caps to be subsequently used in the preservation treatments.

Preservation treatments

1.5 g of inulin and FOS mixtures (5:3 ratio) were added to two out of the three bottles containing 100 mL of strawberry juice. The amount of inulin and FOS incorporated to the juices was selected according to Cassani *et al.* [5] After that, vanillin (1.25 mg mL^{-1}) was added to one out of the two inulin/FOS enriched juices. The juice treated with vanillin was then put into a 100 mL cylindrical vessel (diameter 7 cm; height 7.2 cm) and placed in an inner tank of ultrasound bath cleaner (TestLab, Argentina) of 15×29×15 cm to be sonicated at 20 °C and 40 kHz (power of the ultrasound waves: 180 W transmitted from bottom to above) for 7.5 min in the dark, to avoid any light interference. The operating conditions (vanillin

concentration, ultrasound time and inulin/FOS proportion) were selected after carrying out an optimization study using response surface methodology Cassani *et al*. [17]

Two controls were evaluated to study the effects of enrichment and preservation treatment applied. A batch of sample was enriched with inulin and FOS, as was previously described, but was not submitted to the preservation treatment (enriched control). Another batch of sample was simply strawberry juice without addition of inulin and FOS, or preservation treatment (control).

After applying the mentioned treatments, all the three bottles containing strawberry juices were stored at 5 ºC for 0, 3, 7, 10 and 14 d. At each storage time, 20 mL of juice were collected, frozen for 48 h at -80 ºC and freeze-dried for 48 h at -50 ºC using Mod FD-1A-50 equipment (Boyikang, China). Freeze-dried samples were stored at -20 ºC until HPLC analysis.

Yeasts and molds determination

For the determination of yeasts and molds counts, ten mL of each juice sample were sampled at different times of refrigerated storage (0, 3, 7, 10, 14 d). Serial dilutions (1:10) were carried out in peptonated water (1 g L^{-1}) and surface spread in duplicate. The enumeration of yeasts and molds populations was performed using Yeast-Glucose-Chloramphenicol (YGC, Britania, Argentina) medium, and incubated at 25 ºC for 5 d. Yeasts and molds counts were expressed as log CFU mL^{-1}.

Yeasts and molds experimental data were fitted using the modified Gompertz equations for inactivation and for growth. Inactivation curves were defined according to Eq. 1: [18]

$$\log N = \log(No) + C \times e^{-e^{(B\times M)}} - C \times e^{-e^{B\times(t-M)}}$$

(1)

where N and N_0 are the number of yeasts and molds at time t and at time zero, respectively; M is the time at which the absolute death rate is maximal (d); B is the relative death rate at M; C is the difference in value of the upper and lower asymptote. The negative sign before C means the inactivation of yeasts and molds.

On the other hand, to describe growth curves, the Gompertz equation can be expressed as follows: [19]

$$\log N = \log(No) + A \times e^{-e^{(\frac{\mu max\times e}{A})\times(\lambda-t)+1}}$$

(2)

where N and N_0 are the number of yeasts and molds at time t and time zero, respectively; A is the difference in value of the upper and lower asymptote; μ_{max} is the maximum specific growth rate and λ is the *lag* phase time (d).

The parameters of both Gompertz models were fitted using non-linear regression analysis with the Gauss-Newton estimation method. The goodness of the fit of the model was assessed using root mean square error (RMSE) and evaluating residuals plot.

HPLC Analysis

The HPLC apparatus consisted in HPLC Spectra SYSTEM Isocratic Pump P100 with refractive index detector and a Rheodyne injection valve with a 20 µL-sample loop (Sigma-Aldrich, Missouri, United States, USA). For determination of inulin, FOS, mono and disaccharides, two different columns were used:

(i) Waters Sugar Pak I column (10 µm, 6.5 mm × 300 mm) with Waters Guard Pak LC pre-column inserts (10 µm; Milford, MA, USA). Chromatographic conditions: pump flow rate: 0.5 mL min^{-1}; column temperature: 80 °C; injection volume: 20 µL, and

(ii) Waters Ultrahydrogel Column Linear (10 µm, 7.8 mm × 300 mm) with Ultrahydrogel Guard Column (6 µm, 6 mm × 40 mm) (Milford, MA, USA). Chromatographic conditions: pump flow rate: 0.6 mL min^{-1}; column temperature: 20 °C; injection volume: 20 µL.

The freeze-dried samples were diluted, filtered through 0.45 µm Millipore Durapore membranes (Billerica, MA, USA) and eluted with milli-Q water (mobile phase).

Chromatograms were integrated using WinPCcrom XY 2.0 software (Buenos Aires, Argentina). Standards of fructose, glucose, sucrose, pectin, citric acid, 1-kestose (DP3), nystose (DP4), 1^F-fructofuranosyl nystose (DP5) and inulin were used to determine their retention times and check the linear range of the measurements. Standard stock solutions containing the reference compounds were prepared and diluted appropriately in milli-Q water to construct the calibration curves. Five concentrations of the analyte solutions were injected in triplicate into the HPLC equipment to determine the limits of detection (LOD) and quantification (LOQ). The LOD and LOQ under the present chromatographic conditions were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively.

Statistical Analysis

Results reported in this work are mean three independent values accompanied by their standard errors. Experimental data were analyzed using R, software version 2.12 (R Development Core Team, 2011). Analysis of variance ANOVA ($p < 0.05$) was performed

and Tukey Kramer comparison test was used to estimate significant differences through storage time ($p < 0.05$).

RESULTS AND DISCUSSION

Elution of standards and their separation in strawberry juice samples

The HPLC column used, Waters Sugar Pak I, allowed the separation of monosaccharides (glucose, fructose), sucrose, citric acid and pectin, typically present in strawberry juices. It also allowed the elution of standards of inulin and short chain FOS (DP3, DP4 and DP5). Fig. 1A shows the chromatographic profile of the commercial FOS used in this study. Using the chromatographic profile of the standards, it was possible to separate DP5 (14.65%), DP4 (17.65%), DP3 (28.65%) and sucrose (26.42%) as the main compounds present in the commercial FOS. It is noteworthy the high sucrose content in commercial FOS, which could probably increase the initial contribution of this sugar in the studied juice samples. Glucose and fructose were also present but in much lower concentrations (4.90 and 3.20%, respectively). In addition, two shoulders at 4.99 and 5.33 min (retention time) were observed. Since the Sugar Pak column separates compounds according to their molecular weight, these two peaks could be attributed to DP7 and DP6, respectively. Fig. 1B shows the chromatographic profile of the commercial inulin used in this research employing the same column. A strong peak at 4.94 min (retention time) ascribed to inulin (73.17%), followed by sucrose (9.13%), DP3 (6.41%), DP5 (5.95%) and DP4 (5.34%) was observed.

Waters Sugar Pak I column is packed with a microparticulate cation-exchange resin which allows the resolution of sugars according to their molecular weights and the resolution of organic acids according to their pKa (strength of acids to be dissociated). In the case of sugars, the shorter the retention time, the higher the molecular weight. Regarding organic acids, as they have lower pKa than sugars (they dissociate much easier), they elute together with high molecular weight sugars at the beginning of the chromatographic run. In consequence, in this work the use of this column allowed a good resolution of the carbohydrates and citric acid, usually present in strawberry juices.

Fig. 2 A-C display the chromatograms of all juice samples using the Sugar Pak column. A nice and clear separation of sucrose, glucose and fructose was obtained in all samples studied. In turn, DP3, DP4 and DP5 were clearly separated in those samples in which these compounds were exogenously added (treated sample and enriched control, Fig. 2 B and C). However, inulin could not be resolved as it eluted very close to citric acid and pectin (polysaccharides present also in large amounts in strawberry juices). To clearly separate inulin, the Ultrahydrogel column was used, with milli-Q water as the mobile phase. Fig. 3 A-C display the chromatograms of all juice samples using the Ultrahydrogel column. This column separates macromolecules in aqueous environments based on their hydrodynamic volume and works effectively in a molecular weight range between 10^3 and 10^6. This explains the better resolution of inulin and pectin, two compounds concomitantly present in the fiber-enriched juices investigated in this work. On the contrary, because of the low molecular weight of the remaining compounds, they could not be resolved using this second column (Fig. 3 A-C).

Using both columns enabled the resolution of carbohydrates present in strawberry juices in an easy and environmentally friendly way, as no organic solvents were used.

Linear regression and Limits of Detection (LOD) and Quantitation (LOQ) of the above mentioned analytes are shown in Table 1. A linear behavior was observed in all cases (R^2>0.97), and LODs and LOQs were in the 0.008-0.021 mg mL^{-1} and 0.009-0.110 mg mL^{-1} ranges, respectively (Table 1).

Juices composition immediately after treatments application

As it was mentioned earlier, the main carbohydrates present in strawberry juices were glucose, fructose and sucrose (Fig. 4, time 0). Besides these carbohydrates, inulin and FOS of different DP were identified and quantified in those juices in which these compounds were exogenously added (treated sample and enriched control) (Fig. 4, time 0). Fructose and glucose were the most abundant compounds in all juice samples, with concentrations of 142-197 mg g^{-1} and 144-188 mg g^{-1}, respectively (Fig. 4, time 0). In turn, sucrose concentration was 60 mg g^{-1} for juices enriched with inulin and FOS (Fig. 4A and B, time 0) and significantly lower for non-enriched juices (35 mg g^{-1}, Fig. 4C, time 0). These values were in agreement with those reported by Blanch *et al.* [16] and Macías-Rodríguez *et al.* [7] The higher concentration of sucrose observed in juices enriched with inulin and FOS can be ascribed to the higher concentration of sucrose in FOS and inulin (Fig. 1).

In addition, in those fiber-enriched samples, the initial contents of DP3, DP4 and DP5 were 3.52-4.27, 2.43-3.48, and 2.53-4.38 mg g^{-1}, respectively (Table 3, time 0). Regarding inulin, its initial concentration in both fiber-enriched juices (treated or not with the combined preservation treatment) was not significantly different [Table 3, time 0 (48.75 and 47.50 mg g^{-1} dry matter)]. Thus, it can be concluded that non-thermal preservation treatments did not cleave inulin.

Changes in juice composition during storage

Fig. 4 shows the changes in the mono and disaccharide composition together with the evolution of yeasts and molds counts of strawberry juice samples during storage. According to our previous findings, yeasts and molds represent the dominant spoilage flora of strawberries. [5] Fig. 4A illustrates the inactivation of yeasts and molds in fiber-enriched strawberry juice as a result of the action of vanillin and ultrasound. The experimental values of yeasts and molds survival were adjusted to a non-linear regression, using Gompertz equation for inactivation (Eq. 1). The regression parameters are shown in Table 2. During the *lag* phase (up to day 4), the concentration of sucrose significantly decreased (from 50 to 10 mg g^{-1} dry matter) while the glucose and fructose contents remained unchanged ($p>0.05$) (Fig. 4A). The sucrose consumption can be attributed to the fact that during the *lag* phase, yeasts and molds adapt their metabolism to a new environment rich of nutrients. At the end of the inactivation phase (day 10), a significant increase in the concentration of glucose and fructose was registered, together with a slight increase of sucrose concentration. During the tailing zone (up to day 14), a significant decline of monosaccharides up to the initial levels and a slight decrease of the sucrose concentration were observed. At that moment, the yeasts and molds counts were 2 log CFU mL^{-1}. This result shows the efficiency of the vanillin combined with ultrasound treatment. In this sense, Tomadoni *et al*. [20] reported that when ultrasound treatments are applied in combination with antimicrobials compounds, they improve the efficiency of preservative agents. This phenomenon was ascribed to the high pressure generated during ultrasound treatments that weakens the cell wall, facilitating the penetration of antimicrobial compounds (in this study, vanillin) through the cellular

membrane. Because of their hydrophobic nature, phenolic compounds (including vanillin) preferentially partition into the cytoplasmic membrane, disrupting its integrity and sensitizing the membrane to ultrasound, via interactions with the lipids in the bilayer, membrane-embedded proteins, or both. [21] In addition, Fitzgerald *et al*. [21] reported that low pH (3.25) and low storage temperatures favor the effectiveness of vanillin.

Fig. 4B displays the yeasts and molds growth of strawberry juice enriched with inulin and FOS without preservation treatments. The evolution of yeasts and molds was estimated using Gompertz equation (Eq. 2, Table 2), clearly showing deterioration of juices. During the exponential phase, a significant increase of glucose and fructose at expenses of sucrose was observed. This indicates the hydrolysis of sucrose to give glucose and fructose, as result of the yeasts and molds' metabolism (Fig. 4B). At the end of the exponential phase (up to day 7), a sharp decrease of the monosaccharides concentration was observed but sucrose content remained constant. This can be interpreted considering that sucrose has been already consumed (sucrose concentration during the exponential phase was below 20 mg g^{-1} dry matter) and glucose and fructose are still available for yeasts and molds to grow.

Fig. 4C illustrates the evolution of yeasts and molds in untreated strawberry juices which was also adjusted using Gompertz equation (Eq. 2, Table 2). The increase of glucose and fructose at expenses of sucrose during the exponential phase indicates, also in this case, the consumption of sucrose by yeasts and molds. It is worth to mention that in this group of samples, the sucrose concentration decreased up to 4 mg g^{-1} dry matter, a value very close to 0 (Fig. 4C). This could be related with the lower concentration of sucrose at time 0 in this group of juices. At the end of the exponential phase (up to 10 days of storage), the concentration of monosaccharides and sucrose remained constant because there was no more sucrose available to be hydrolyzed (Fig. 4C). Finally, during the stationary phase a sharp

decrease of the fructose and glucose content was observed. This can be interpreted considering that at that moment, the yeasts and molds counts were high (8 log CFU mL^{-1}) and high amounts of simple sugars were consumed to produce metabolites, such as ethanol, lactic or acetic acids, as reported in Ragaert *et al.* [22]

Similar results were found by Mtaoua *et al.* [23], who observed an increase on soluble solids content of date juice processed by high-intensity pulsed electric fields after the first week of storage, and then a decrease of its content after five weeks of refrigerated storage. They also attributed the decrease in ºBrix to microbial deterioration, especially, on the untreated juice, which spoiled after two weeks of storage. In addition, Wang *et al.* [24] studied the effect of storage time and temperature on simple sugars of concentrated carrot juice, ascribing the observed decrease of sucrose to its inversion into glucose and fructose at low pH.

The parameters of Gompertz models, fitted using non-linear regression analysis with the Gauss-Newton estimation method are shown in Table 2. Their goodness and accuracy was estimated by determining the root mean square error (RMSE) (Table 2). The lower the RMSE values the better the fitting. [25] A good fitting was observed for all growth and survival yeasts and molds' curves analyzed in this work (Table 2). Any model intended to interpret microorganisms' growth or inactivation should provide parameters allowing the interpretation of different treatments. In this study, inactivation curves were modeled for samples preserved using non-thermal treatments and growth curves, for the non-treated ones.

Among samples in which no preservation treatment was applied (with or without inulin and FOS), the main parameters fitted (μmax, λ and A) were similar. The parameter A represents the maximum growth obtained and was about 3 log for both samples, indicating that yeasts and molds increased 3 log-decimal up to the end of storage. In the same way, the

maximum growth rate and the *lag* time were similar in both samples (Table 2). This was an expected result since in a previous study, Cassani *et al*. [5] found that the incorporation of inulin and FOS to strawberry juices does not have any significant effect on yeasts and molds counts. In turn, the growth rate (□) was similar for both samples and also comparable with the parameter B (relative death rate) of samples treated with vanillin and ultrasound (0.46) (Table 2). This indicates that the inactivation kinetics of yeasts and molds in preserved samples was similar to the growth kinetics of untreated ones.

Another test to judge the reliability of the Gompertz model is the residual plot examination. Residuals are the differences between the values of the observed dependent variable and the values predicted by the model. The residual analysis is useful to confirm whether a model is confident for the data analyzed or not. [26] Fig. 5 shows the residuals obtained from the survival curves of the three groups of strawberry juices. Residuals were distributed randomly and they fell within a horizontal band centered around 0, displaying no systematic tendencies to be positive or negative. When residual versus fitted values plot (Fig. 5) was examined, it can be concluded that the Gompertz models were adequate to describe the data analyzed.

To evaluate the stability of FOS and inulin added to the juices treated or not with vanillin and ultrasound (treated sample and enriched control), the concentration of these compounds was determined during storage at 5 °C (Table 3). In fiber-enriched samples treated with ultrasound and vanillin, the concentration of DP3 and DP4 did not show significant changes up to the 10^{th} day of storage. Only after day 14, a significant decrease was observed (Table 3). In addition, no significant changes were observed in the concentration of DP5 up to 14 days of storage. In turn, samples not treated with vanillin and

ultrasound showed no significant changes in the concentration of DP3 and DP4 but the concentration of DP5 increased at day 7 and noticeable decreased after that day.

Regarding inulin, samples preserved with non-thermal techniques showed no significant changes during the whole storage period. On the contrary, a sharp decrease of the inulin concentration was observed in non-treated samples. The hydrolysis of inulin can be attributed to acidification resulting from the growth of yeasts and molds in untreated samples (Fig. 4B). These conditions had a direct effect on the cleavage of the inulin chain into FOS of lower DP. These FOS can be produced from oligomers of higher DP because these latter compounds have a relatively high content of terminal fructosyl units. [27]

In this work, the concentrations of FOS and inulin added to the juices were defined considering a typical human intake of strawberry juice (200 mL d^{-1}). However, the chemical hydrolysis of FOS and inulin is easier than that of other oligosaccharides at low pH, which are the conditions of different food matrices (*i.e.*, juices) (pH < 4.6). Some authors have reported that inulin and FOS readily hydrolyze under low pH and temperature conditions. [26] Therefore, for these prebiotics to serve as functional food ingredients, they must be chemically stable to food processing treatments, such as heat, low pH, and Maillard reaction conditions. [28] In this regard, Vega and Zuñiga-Hansen [27], investigated the stability of FOS in orange and tomato juices (pH 3). In this work, fructans' levels were monitored during refrigerated storage to assess if any partial hydrolysis occurred. It is worth noting that inulin and DP5 added to strawberry juices preserved with vanillin and ultrasound were not degraded neither during the storage time nor at the low pH of strawberry. These results show that the application of non-thermal techniques ensured the stability of inulin and FOS during storage. These findings are consistent with Rößle *et al*. [29] who reported no significant hydrolysis during storage of fresh-cut apple wedges containing commercial prebiotics.

Based on the results obtained in this work, it can be concluded that adding FOS and inulin to strawberry juices can be a good strategy to functionalize them.

CONCLUSION

The application of non-thermal techniques, such as vanillin and ultrasound, resulted an efficient strategy to control the growth of yeasts and molds in strawberry juices enriched with FOS and inulin. Hence, combining prebiotics (FOS and inulin) with non-thermal and environmentally friendly preservation treatments (vanillin and ultrasound) resulted in an adequate strategy to obtain safe and shelf-stable strawberry juices that could appeal to health awareness consumers concerned about human nutrition in the context of the current dietary guidelines.

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TABLES

Table 1. Linear regression and Limits of Detection (LOD) and Quantitation (LOQ) of fructans and carbohydrates.

Analyte	Regression equation	R^2	Linear range (mg mL^{-1})	LOD (mg mL^{-1})	LOQ (mg mL^{-1})
Glucose	$Y = 6 \cdot 10^7 \ x + 3 \cdot 10^6$	0.97	0.25-1	0.034	0.110
Fructose	$Y = 7 \cdot 10^7 \ x$ - 573898	0.99	0.25-1	0.033	0.110
Sucrose	$Y = 7 \cdot 10^7 \ x - 10^6$	0.97	0.057-0.228	0.021	0.071
DP3	$Y = 7 \cdot 10^8 \ x - 5 \cdot 10^6$	0.99	0.02-0.093	0.008	0.028
DP4	$Y = 5 \cdot 10^8 \ x - 2 \cdot 10^6$	0.98	0.02-0.093	0.020	0.08
Inulin	$Y = 3 \cdot 10^7 \ x + 9 \cdot 10^6$	0.97	0.25-1	0.002	0.009

Table 2. Gompertz model parameters and mean square error for each strawberry juice sample

TREATMENTS				
Samples + fiber + preservation treatment (Figure 3A)				
M	**B**	**C**	**No**	**RMSE**
7.58	0.46	3.26	194984.46	0.029
Samples + fiber without preservation treatment (Figure 3B)				
A	**μmax**	**λ**	**No**	**RMSE**
3.05	0.43	0.36	144495.35	0.023
Samples having neither fiber nor preservation treatment (Figure 3C)				
A	**μmax**	**λ**	**No**	**RMSE**
3.47	0.40	0.25	97261.47	0.041

M is the time at which the absolute death rate is maximal (day); **B** is the relative death rate at M; **C** and **A** are the difference in value of the upper and lower asymptote; **No** is the number of yeast and mold at time zero; **μmax** is the maximum specific growth rate; **λ** is the lag phase time (day).

Table 3. Evolution of the FOS and inulin concentrations (mg g^{-1} dry matter) during storage of strawberry juices at 5 ºC.

Analyte	Time of storage (days)				
	0	3	7	10	14
			Treated sample		
DP3	3.52±0.12^{ab}	3.42±0.26^{bc}	3.51±0.05^{ab}	4.26±0.09^{a}	2.63±0.01^{c}
DP4	2.43±0.17^{ab}	3.19±0.48^{ab}	2.50±0.44^{ab}	3.65±0.09^{a}	1.88±0.01^{b}
DP5	4.38±0.06^{a}	2.66±0.07^{a}	2.55±0.66^{a}	2.84±0.09^{a}	3.63±0.32^{a}
inulin	48.75±1.25^{a}	ND	50.00±0.00^{a}	ND	43.75±4.73^{a}
			Enriched control		
DP3	4.27±0.07^{a}	4.09±0.33^{a}	2.97±0.14^{a}	2.98±0.35^{a}	3.59±0.17^{a}
DP4	3.48±0.29^{a}	3.00±0.05^{ab}	1.97±0.04^{b}	3.04±0.34^{ab}	3.26±0.16^{a}
DP5	2.53±0.29^{b}	3.79±0.19^{b}	5.49±0.30^{a}	2.29±0.14^{b}	2.20±0.48^{b}
inulin	47.50±2.50^{a}	ND	46.66±3.33^{a}	ND	25.41±3.14^{b}

Data are shown as means ± standard deviation of three determinations. Values with different letters in the same row indicate significant differences ($p<0.05$) during storage time. **Treated sample**: strawberry juice enriched with inulin and FOS and treated with vanillin and ultrasound; **Enriched control**: strawberry juice enriched with inulin and FOS without preservation treatment. **DP3**: 1-kestose; **DP4**: nystose; **DP5**: 1^{F}-fructofuranosyl nystose. **ND**: Not determined.

FIGURE CAPTIONS

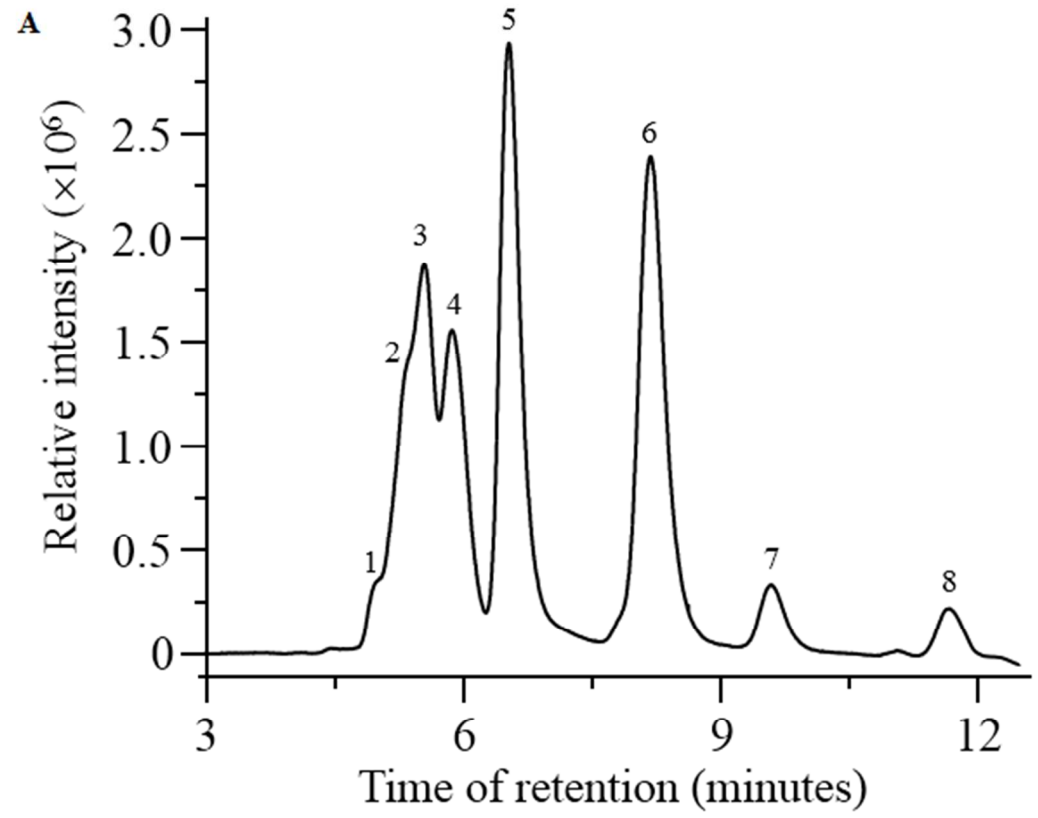
Fig. 1 (A) HPLC with RI chromatogram profile of commercial FOS using the Sugar Pak Column. Peaks: 1, unidentified compound; 2, unidentified compound; 3, 1^F-fructofuranosyl nystose (DP5); 4, nystose (DP4); 5, 1-kestose (DP3); 6, sucrose; 7, glucose; 8, fructose. (B) HPLC with RI chromatogram profile of commercial inulin using the Sugar Pak Column. Peaks: 1, inulin; 2, 1^F-fructofuranosyl nystose (DP5); 3, nystose (DP4); 4, 1-kestose (DP3); 5, sucrose.

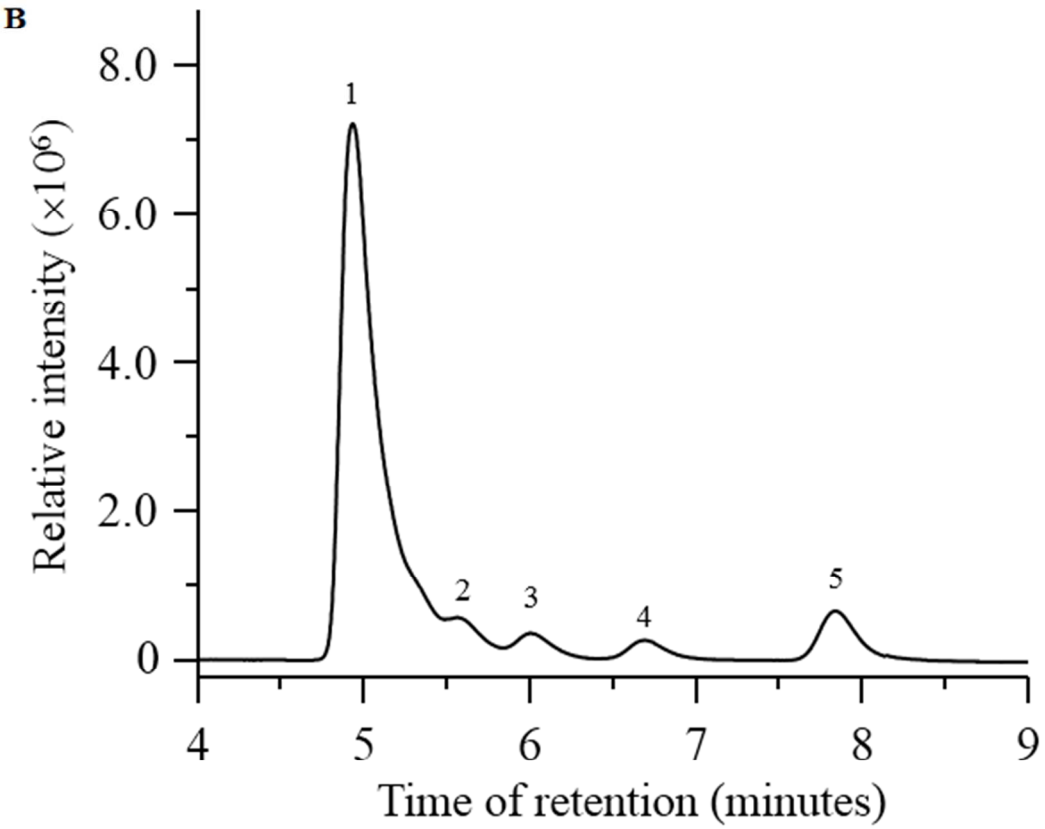
Fig. 2 HPLC (refractive index detector) chromatograms obtained with a Sugar Pak column (A) non-enriched strawberry juice without preservative treatments (peaks: 1, inulin, pectin and citric acid; 2, sucrose; 3, glucose; 4, unidentified compound; 5, fructose). (B) strawberry juice enriched with inulin and FOS without preservative treatments (peaks: 1, inulin, pectin and citric acid; 2, 1^F-fructofuranosyl nystose (DP5); 3, nystose (DP4); 4, 1-kestose (DP3); 5, sucrose; 6, glucose; 7, unknown sugar; 8, fructose); (C) strawberry juice enriched with inulin and FOS and preserved with vanillin and ultrasound (peaks: 1, inulin, pectin and citric acid; 2, 1^F-fructofuranosyl nystose (DP5); 3, nystose (DP4); 4, 1-kestose (DP3); 5, sucrose; 6, glucose; 7, unidentified compound; 8, fructose).

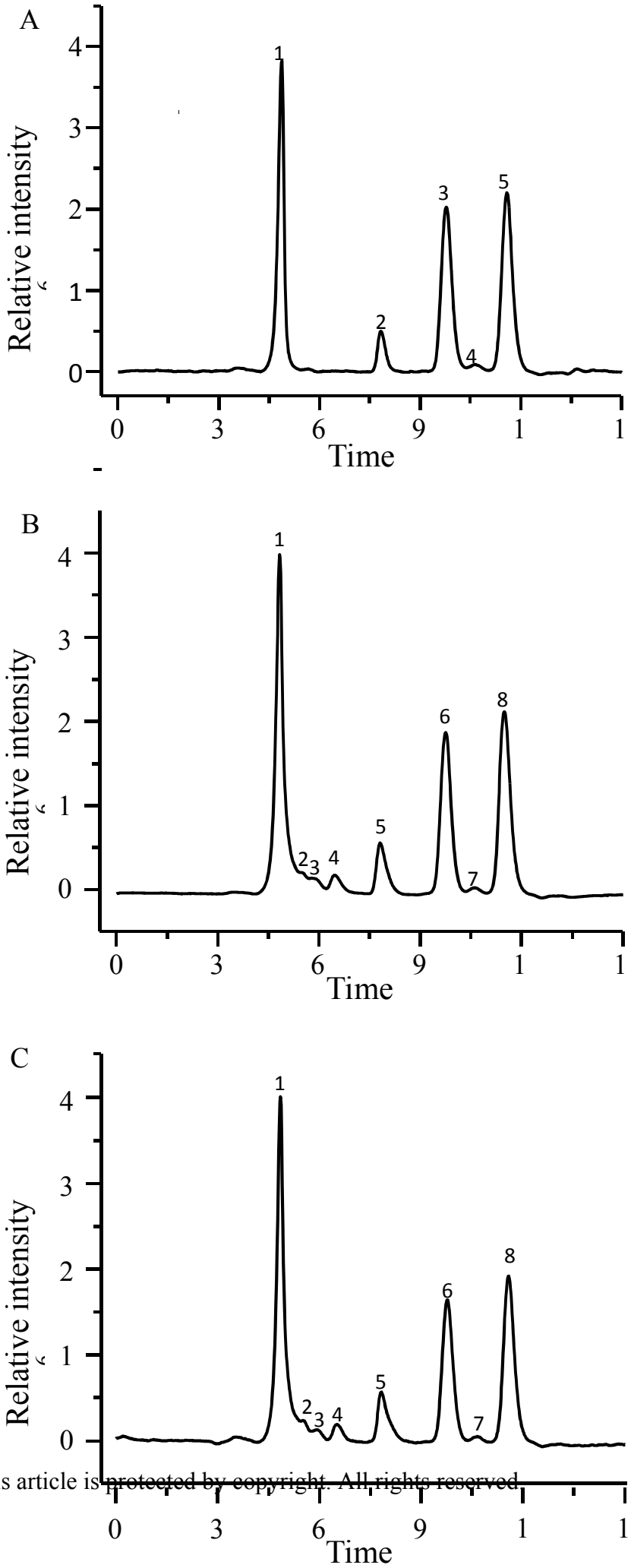
Fig. 3 HPLC (refractive index detector) chromatograms obtained with a Ultrahydrogel column (A) non-enriched strawberry juice without preservative treatments (peak: 1, pectin); (B) strawberry juice enriched with inulin and FOS without preservative treatments (peaks: 1, inulin; 2, pectin); (C) strawberry juice enriched with inulin and FOS and preserved with vanillin and ultrasound (peaks: 1, inulin; 2, pectin).

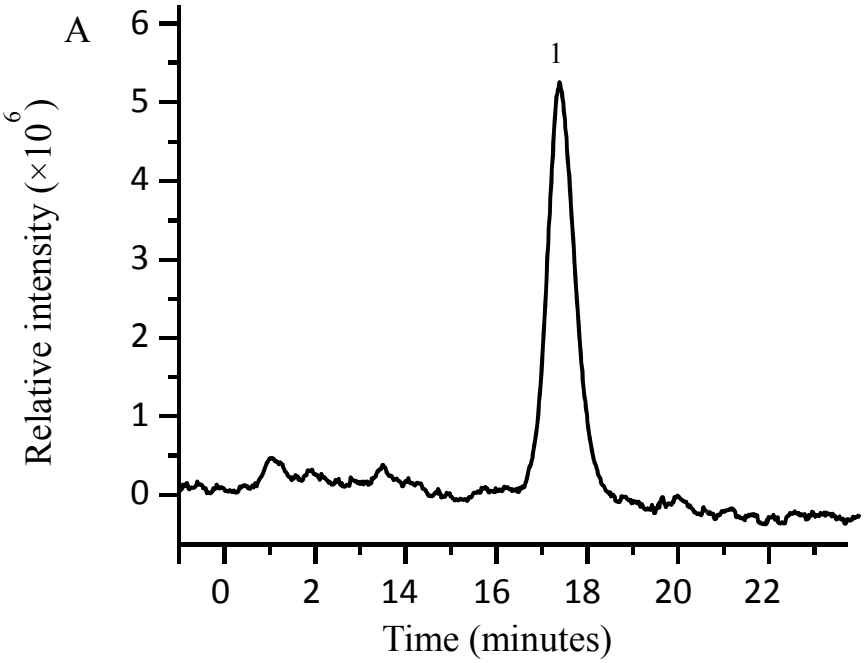
Fig. 4 Evolution of concentration of sugars [(■) glucose, (♦) sucrose, (▲) fructose], survival curve (●) and estimated curve via Gompertz model on: (A) strawberry juice enriched with inulin and FOS and preserved with vanillin and ultrasound, stored at 5 ºC for 14 d (---); (B) strawberry juice enriched with inulin and FOS without preservative treatment, stored at 5 ºC for 14 d (---); (C) non-enriched strawberry juice without preservative treatments, stored at 5 ºC for 14 d (---).

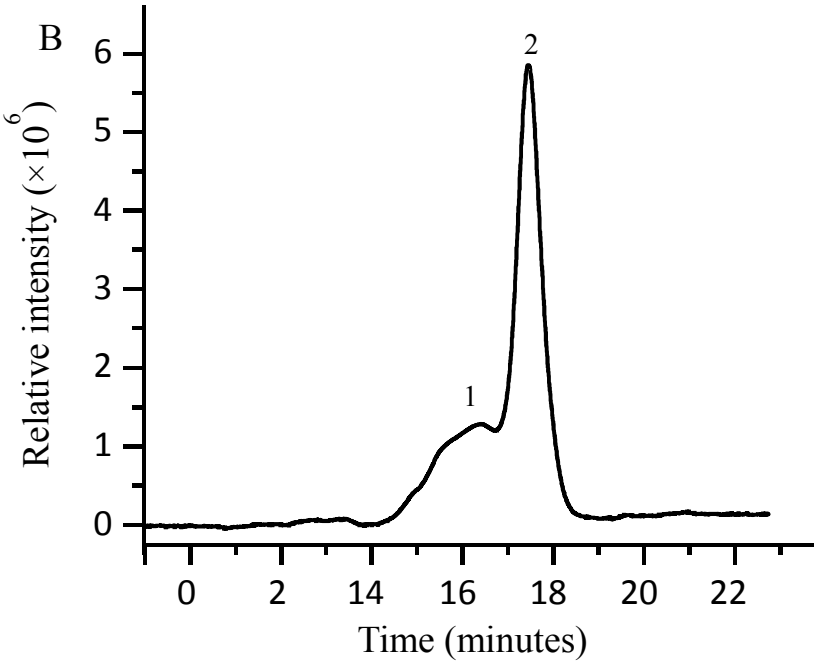
Fig. 5 (A) Residual analysis of Gompertz models for: (A) the yeasts and molds inactivation of strawberry juice enriched with inulin and FOS and treated with vanillin and ultrasound; (B) the yeasts and molds growth of strawberry juice enriched with inulin and FOS without preservation treatments; (C) the yeasts and molds growth of strawberry juice without inulin and FOS added and without preservation treatments.

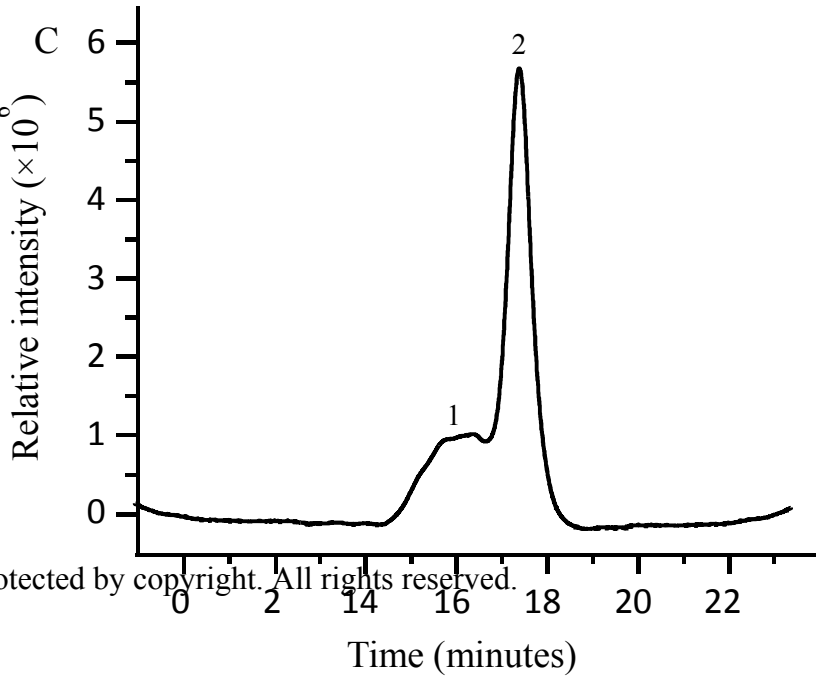












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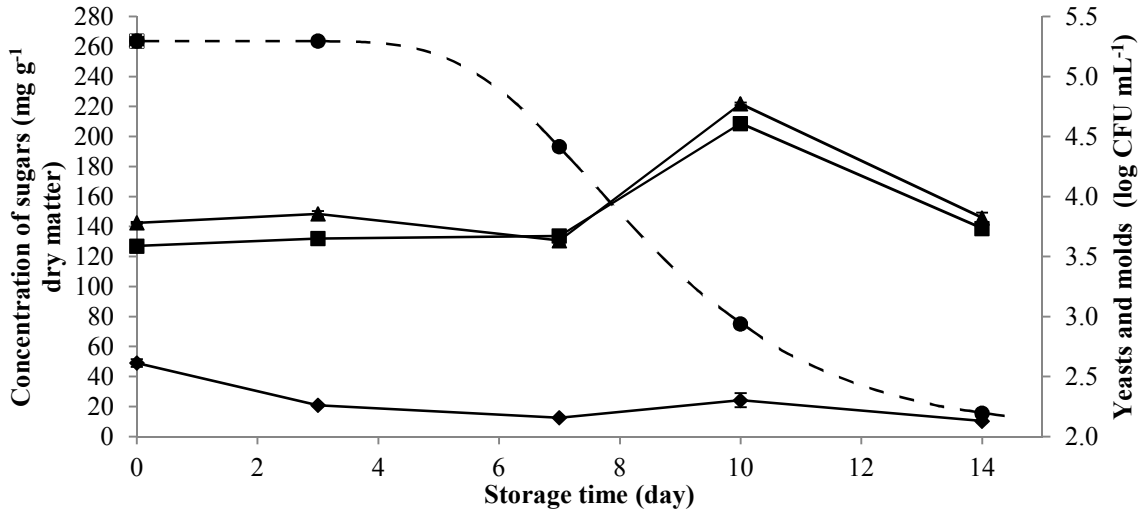

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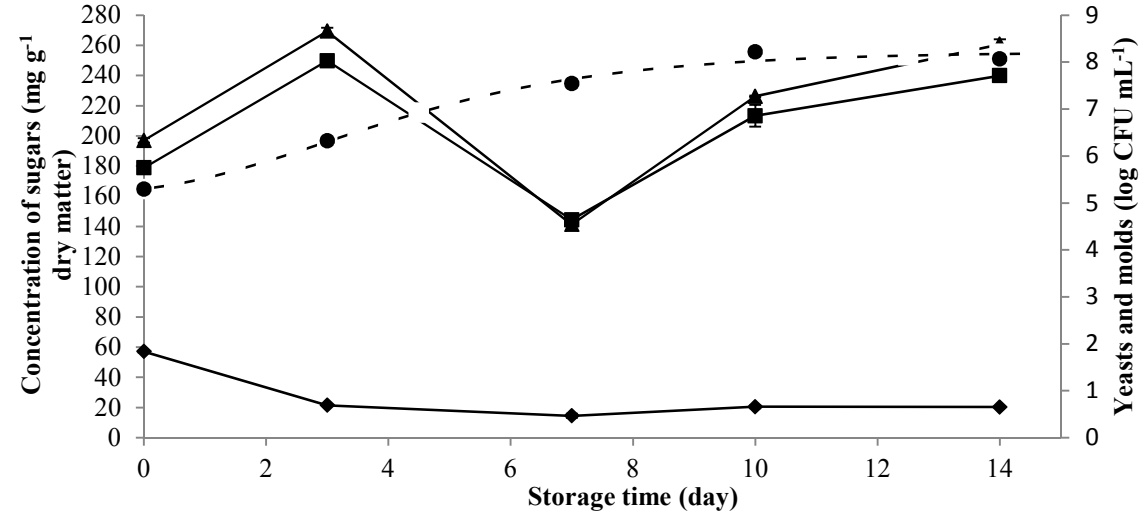

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