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# Storage Stability Test of Sweet Sorghum SPV 422 (Sorghum bicolor L. Moench) Syrup



#### **ABSTRACT**

A four-month storage stability test on the sweet sorghum (SPV 422) syrup was conducted. The syrup's initial Brix, storage containers' material of construction and incubation temperature were the experimental parameters. A higher degree Brix concentration of around 40-65 degree Brix is preferred due to having least reductions in the reducing sugar content, pH and dissolved solids concentration upon storage. This study also confirmed that high density polyethylene (HDPE) was a more suitable material than carbon steel for storage containers of sweet sorghum syrup. On the other hand, analyses of the dependence of the syrup's stability on storage temperature proved that a higher temperature 40°C was more preferred for lesser degree of changes in amount of reducing sugars and Brix. Conversely, a larger drop in pH was observed at 40°C incubation temperature.

Key words: Brix, reducing sugar, storage stability, sweet sorghum

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#### **INTRODUCTION**

Several studies on the viability of sweet sorghum as a feedstock for bioethanol production have been conducted in the Philippines. However, after several investigations on the potential of sweet sorghum as a bioethanol feedstock, local manufacturers are still apprehensive to utilize sweet sorghum. This may be due to the fast deterioration of crop's fermentable sugars and its short harvest window (*Kumar et al 2010*; *Wu et al 2010*). One suggested solution is to preserve the extracted juice by concentrating it into syrup. Concentrating the juice through evaporation helps reduce the susceptibility of the syrup to microbial activity (*Pin et al 2011*). However, evaporation requires a relatively high amount of energy; therefore, it is necessary to determine the minimum concentration for storage prior to use.

Along with the initial concentration, other physicochemical properties may also affect the storage failure time of the syrup (*Guillet and Rodrigue 2010*). Thus, this study also considered other parameters like the storage temperature and the type of material of the storage containers. Among all environmental factors, these two greatly affect the rate of deterioration, quality and safety of any type of product (*Lee 2010*).

In general, this is a study on the shelf life analysis of sweet sorghum (SPV 422) syrup based on the initial Brix, storage temperature and the containers' material of construction. Specifically, this study identified how these

parameters interact and affect the stability of the syrup's pH, reducing sugar concentration and Brix.

The storage stability of sweet sorghum syrup was monitored for four months. The experiments were conducted at the laboratory of the Department of Chemical Engineering, CEAT, UPLB. Sugar profiling was obtained by measuring the total soluble solids (Brix) and reducing sugars of the samples. On the other hand, acidity was monitored by measuring the pH of the medium every two weeks.

#### MATERIALS AND METHODS

#### **Preparation of Sweet Sorghum Syrup**

Sweet sorghum stalks were harvested from a plantation located in Sagay City, Negros Occidental, Philippines. The stalks were milled at Organic Producers in the Island of Negros Multi-Purpose Cooperative (OPTION-MPC) plant. OPTION-MPC sugar mill is owned and operated by sugarcane planters of Sagay City, Negros Occidental. The extracted juice was hauled to San Carlos Bioenergy, Inc. (SCBI), a company which manages an integrated bioethanol distillery and power cogeneration facility. The sweet sorghum juice was procured by the Department of Chemical Engineering, CEAT, UPLB from SCBI on May 25, 2012. The juice was clarified and then concentrated to 65°Brix syrup through evaporation. The syrup was

analyzed for fermentable sugars, reducing sugars, Brix (i.e., % soluble solids) and sugar determined by polarimetry.

### **Syrup Storage Experimentation**

The parameters considered in this study were the syrup's initial solid concentration expressed in Brix, the type of material used for syrup's storage containers, and the storage temperature. Sorghum syrup's initial solid concentrations were varied by dilution using water. Concentrations were set to 29°, 41°, 54° and 65°Brix. The syrup with pre-adjusted solid concentration was poured in sterilized containers made of either carbon steel (CS) or high density polyethylene (HDPE). CS containers were sterilized using a convection oven at a temperature of 115°C for about 2 hrs. HDPE bins, on the other hand, were decontaminated via hot water splashing, because these containers could not be autoclaved. The bins containing the sweet sorghum syrup were enclosed in a temperature controlled incubator. Syrup was stored at temperatures of 30°, 35° and 40°C for a period of four months.

#### **Analytical Methods**

Including time 0, sampling was conducted periodically every two weeks. Sample syrups' pH, Brix, and reducing sugar contents were determined. Responses such as pH and Brix were obtained using a pH meter and a portable Brix refractometer, respectively. Reducing sugars were determined using Miller's modified dinitrosalicylic (DNS) analysis (*Miller 1959*).

The experimental design used in this study was a three-factor factorial with subsampling and repeated measures. Gathered data were assessed using Scheffe and Tukey's method with inclusion of Repeated Measures Analysis of Variance (ANOVA). Parametric effects were analyzed statistically at 5% level of significance.

#### RESULTS AND DISCUSSION

### Characterization of Sweet Sorghum (SPV 422) Syrup

An amount of 46.87 m³ of sweet sorghum syrup had a corresponding mass of 61.78 t and had a specific gravity of 1.32 (**Table 1**). The percent dissolved solids (Brix) were 65.14. This Brix corresponds to dissolved solids of sucrose, monosaccharides, ash and other organic substances (*Rein 2007*). The apparent sugar content (Pol) of the entire sample was 31.92%. The resulting apparent purity [(Pol/Brix) x 100] was 49%. Syrup's glucose content was 17.17% by mass of the sample. The syrup was made of 50.77% total reducing sugars. Hence, aside from glucose, the syrup

Table 1. Selected physico-chemical properties of sweet sorghum syrup (SPV 422) produced from stalks harvested from a plantation in Sagay City.

% Fermentable Sugars	49.59		
∘Brix	65.14		
Pol	31.92		
Apparent Purity	49.00		
Specific Gravity	1.32		
% Reducing Sugars	17.17		
Volume, m <sup>3</sup>	46.87		
Mass, ton	61.78		

contained other types of reducing sugars such as xylose, ribose, arabinose, fructose, sorbose, galactose, and mannose (*FAO n.d.*). The fermentable sugars were 49.59% of the sample. The value mostly accounts for readily fermentable reducing monosaccharides such as glucose and fructose, and the non-reducing disaccharide sucrose.

The sugar content and soluble solids of the juice are dependent on both the maturity stage when the crop is harvested and on the postharvest conditions (Mamma et al. 1996 and Phowchinda et al. 1997). Based on the study by Kumar et al. (2013), the overall mean of total soluble solids (Brix) of most genotypes of sweet sorghum was highest when the harvested sorghum was already physiologically mature. The marginally high value of Brix was indicated by rapid accumulation of sucrose until the development of the panicle of the sweet sorghum. Hence, Brix has a direct proportionality with sucrose. This is in agreement with the definition of Brix cited by Wortmann et al. (2010), stating that Brix for sugar syrups like with sweet sorghum, is a measure of dissolved sugar to water mass ratio. Since sucrose is the major sugar in the juice, any incremental rise in the sucrose content is usually attributed to an increase in Brix

Gains and losses with the sugar content of sweet sorghum were also influenced by the postharvest conditions. Since weather conditions such as high temperature and humidity have a great impact on stalk deterioration in the tropics, the time lag between harvesting to milling of the stalks must be minimized. If not prevented, microbial contamination tends to induce acid inversion of sucrose. The inversion hydrolyzes the sucrose into monosaccharides, glucose and fructose by the acid invertase secreted by the yeasts (*Rao et al. 2012*).

# Storage Stability Dependence on Syrup's Initial Concentration

The influence of initial concentration (i.e., Brix) on

the changes in Brix upon storage, amount of reducing sugars and pH was determined.

**Effect on Brix**. Upon storage of the syrup for four months, a drastic decrease on dissolved solids of the 29°Brix and 41°Brix syrup samples was observed (Figure 1), constituting up to 36.4035% and 30.3030%, respectively. The concentration of the 53°Brix dropped by 7.8788%; while the Brix reading for the undiluted syrup decreased by only 4.1995%. Comparative stability of the 65°Brix syrup could possibly be due to its lesser water content. Water is required by microorganisms to grow and reproduce. Water supports most biochemical reactions by bacteria and yeasts (Childs and Chabot 2007), therefore syrups with higher water content are more prone to spoilage. In addition, contaminants during proliferation consume the solids which are mostly sugar as substrate for population growth (Rao et al. 2012). Hence, a greater amount of water in the syrup may correspond to a higher degree of microbial growth and a larger amount of solids for consumption.

Effect on Reducing Sugar Concentration. The reducing sugar profile of the sweet sorghum syrups at varying initial Brix concentrations illustrates a decrease in the reducing sugars of all tested syrups from the 4th week until the 8th week, then a sudden rise in the amount of reducing sugars during the 10th week (Figure 2). The trend is followed by a partially steady decrease in the reducing sugar concentrations up to the last week. Similar results were shown by a study of Wu et al. (2010). With sugar profiling of sweet sorghum juice stored at ambient conditions, 20% of the fermentable sugars tend to deteriorate in the first period (Wu et al. 2010). This may be caused by initial activities of the contaminating organisms. At the second stage of storage, the syrup was still chemically unstable.

70 60 50 oBrix 30 20 10 0 0 2 8 10 12 14 16 18 Time, weeks → 29 Brix → 41 Brix → 53 Brix → 65 Brix

Figure 1. Mean Brix profiles of sweet sorghum syrup samples of varying initial concentrations.

Sucrose, a non-reducing sugar may have been inverted to reducing sugars, glucose and fructose, resulting to the trend observed during the 10th week (**Figure 2**). A decrease in reducing sugars is evident until the 16th week may be due to spoilage microorganisms plaguing the syrup and alongside consuming the sugars, as exhibited by the formation of green mycelium on the surface of the syrup.

On the average, the reducing sugar concentration of each syrup sample significantly decreased per week. From the initially 29°Brix syrup, 44.2% of the reducing sugars during the 4th week was lost (**Figure 2**). Based on the results, minimum Brix of the sample must be set at 41° to prevent significant loss of reducing sugars for a longer period of time. This may be the reason why a large scale bioethanol plant in Isabela, Philippines, built and operated by Green Future Innovations, Inc. (GFII), evaporates its sugarcane juice to only 40°Brix prior to ethanol fermentation (*GFII* 2013).

Effect on the Syrup's pH. The accumulation of organic acids in the sweet sorghum syrup is also an indicator of storage instability. A decrease in pH corresponds to an increase in formation of organic acids in the syrup (*Mazumdar et al. 2012*). Acid generation in the syrup is unwanted because it initiates sucrose inversion (*Rao et al. 2012*).

During the four-month storage stability experiment, a drastic drop in pH of four tested syrups was observed in the 2nd week (**Figure 3**). However, the pH values were relatively unchanging for all tested syrups in the succeeding weeks, indicating that the effect of pH on the storage stability of the syrup is statistically insignificant (**Figure 3**). This is the same phenomenon based on the study

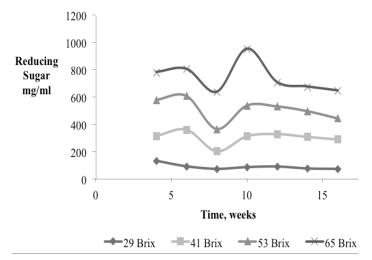


Figure 2. Reducing sugar profiles of sweet sorghum syrup samples of varying initial concentrations.

of Wu et al. (2010). During the first two weeks, a greater amount of organic acids may have been possibly secreted by contaminating microorganisms. Upon reaching the optimum pH in which the microorganisms can still survive, the formation of organic acids stopped and competition for substrate (i.e., syrup sugars) consumption began, leading to a gradual decline in pH values.

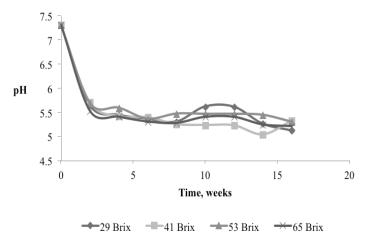


Figure 3. Average pH profiles of sweet sorghum syrup samples of varying initial concentration stored in HPDE containers at 40°C.

Table 2. Approximate minimum pH and temperature limits for microbial growth.

Organism	Lowest	Lowest
	pН	Temperature
	Limit	Limit (°C)
Bacteria		
Bacillus cereus (mesophilic)	4.9	10
Bacillus cereus (psychrotrophic)	4.9	5
Brochothrix thermosphacta	4.6	0
Campylobacter spp.	4.9	30
Clostridium botulinum	5.0	3.3
(nonproteolytic)		
Clostridium botulinum (proteolytic)	4.6	10
Clostridium perfringens	4.5	5
Escherichia coli	4.4	7
Lactobacillus spp.	3.0	4
Listeria monocytogenes	4.3	0
Most lactic acid bacteria	3.5	5
Pseudomonas spp.	5.0	-2
Salmonella spp.	4.0	5
Staphylococcus aureus	4.0	7
Molds		
Aspergillus flavus	2.0	3
Most molds	1.5	<0
Yeasts		
Most yeasts	1.5	-5
Saccharomyces cerevisiae	2.3	0

Source: Leistner and Gould 2002

The pH of 41°Brix syrup did not significantly vary with the 65°Brix syrup during the 4th, 6th, 8th and 16th weeks (**Figure 3**). After four months of storage, the pH of 41 °Brix, 53°Brix and 65°Brix syrups decreased by 27.1689%, 27.3973% and 28.6530%, respectively. The largest pH drop of 29.9086% was observed with the 29°Brix syrup. It may be concluded that the pH stability depends on the presence and on the type of microorganisms in the syrup. Every microorganism has its own pH limits in order to reproduce and survive (*Leistner and Gould 2002*) (**Table 2**).

# Significance of Containers' Material of Construction on Syrup Stability

Similar to the importance of food packaging, the type of material with which the containers were constructed had also an effect on the storage stability of the syrup. In this study, the syrup bins were made of either CS or HDPE. The effect of material of construction on the stability of the sweet sorghum syrups of varying Brix concentration, amount of reducing sugars and pH was investigated.

Effect on Brix. In terms of the effect on Brix, the Brix of the samples stored in CS containers differed significantly to that of the syrup samples stored in HDPE (Figure 4). At the 2nd week, Brix of the syrups stored in HDPE was lower than with that of syrups in CS containers. After two succeeding weeks, Brix readings on the syrups in both types were similar. Then, in the remaining 12 weeks, a faster rate of deterioration of soluble solids was observed on syrups in CS bins (i.e. 19.4590%) than with syrups in HDPE containers (i.e. 12.6963%). This shows that HDPE is a better material for storage containers despite the fact that sugar producers commonly use CS for their equipment. Possible corrosion and abrasion of carbon steel may cause instability during storage (Fechter 2009). Since the

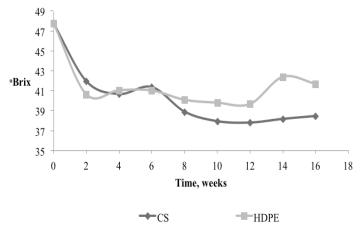


Figure 4. Mean Brix profiles of the sweet sorghum syrup samples stored in carbon steel (CS) and HDPE containers at 40°C.

syrup is an organic substance, its composition can easily be disrupted with carbon from the container (*US EPA n.d.*). This is referred to as oxidation. Upon oxidation, the inner surface of the container interacting with the syrup is considered a chemically and biologically active layer. On this corrosive layer, the syrup turns out to be more acidic and rich in moisture content, making the environment more susceptible to microbial growth. Consequently, a higher rate of microbial growth means a higher degree of substrate (i.e., sugars in dissolved solids) consumption.

Effect on Reducing Sugar Concentration. The mean reducing sugar profiles of sweet sorghum syrup samples stored in CS and HDPE containers were also studied. Similar to the previous plot, HDPE is ideal to be used as a storage material than CS (Figure 5). Statistically, the amount of reducing sugars in syrups stored in two types of containers were different at all periodic readings except in the 14th week. It was found that a significantly higher reducing sugar loss was recorded in samples stored in CS containers than those in HDPE.

Sugars may be considered as reactive oxygen species. They predominantly exist in an open chain form in which the carbonyl is left unprotected from oxidation. Sugar oxidation starts with tautomerization of the open chain form of aldoses or ketoses to corresponding enediols. Enediols, then, produce radicals through sequential one-electron oxidation. These radicals are superoxides in form which are vulnerable to attack by contaminating microorganisms (*O'Brien and Bruce 2010*).

Effect on the Syrup pH. The plot of the average pH profile of the syrup samples stored in CS and HDPE containers showed that syrup samples in HDPE vessels turned out to be more acidic than those in CS containers starting with the 2nd week (Figure 6). A change in pH in a lesser degree

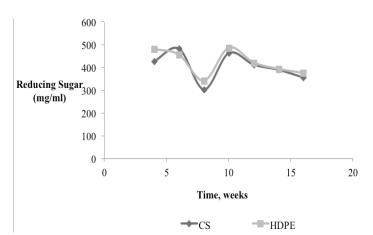


Figure 5. Average reducing sugar profiles of the sweet sorghum syrup samples stored in carbon steel (CS) and HDPE containers at 40°C.

should be observed upon using HDPE containers since HDPE is considered as a relatively inert material (*US EPA n.d.*). A larger pH drop in the syrup samples stored in HDPE may be due to by-product inhibition. Organic acids tend to act as by-products which if present in excess amount decelerate the metabolic activities of existing spoilage microorganisms, further resulting to a lesser degree of substrate deterioration (*Lee 2001*). The dissolved solids and the reducing sugars at this point were considered as the substrates. This may be the reason for a relatively lower decrease in both Brix and reducing sugar concentrations for HDPE containers than with that of CS (**Figures 4** and **5**).

## **Effect of Incubation Temperature on Syrup Stability**

Freezing or refrigeration is typically well known as a form of preservation, but this idea seems not suitable for sugar juice. According to *Bridgers et al.* (2011), freezing of sugar types of juice decreased the sucrose concentration and increased the fractions of both glucose and fructose. Therefore, freezing temperature should not be always regarded as ideal for efficient storage of sweet sorghum syrup.

In this experiment, storage temperatures were set at 30°, 35° and 40°C to determine the potential of storing the syrup samples at about room temperature. However, the overall result of this test on the storage stability showed that a room temperature of 30°C was not suitable.

Effect on Brix. Minimal changes in Brix were observed in syrups stored at 35° and 40°C (Figure 7). Usually at 30°C, two common spoilage microbial species thrive best to contaminate the syrup, *Leuconostoc mesenteroides* and *Lactobacillus plantarum* (*Daeschel et al. 1981*). Other microorganisms can survive at this temperature (Table 2).

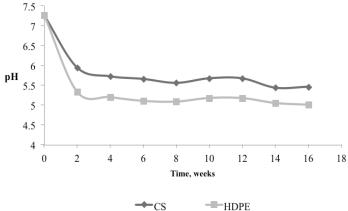


Figure 6. Average pH profiles of 65° Brix sweet sorghum syrup samples stored in carbon steel (CS) and HDPE containers at 40°C.

Effect on Reducing Sugar Concentration. Similar to Figure 7, syrup samples stored at 30°C had a larger difference (27.0151%) in reducing sugar content 12 weeks after the 3rd reading (Figure 8). This is followed by the samples stored at 35°C, having a 15.9534% decrease in the reducing sugar concentration. On the other hand, syrups stored at 40°C had the least drop in the amount of reducing sugar reaching up to only 15.8155%. The trend observed on the effect of storage temperature on the reducing sugar concentration profile agrees with that of the Brix profile due to the linear relationship between reducing sugar and Brix concentrations as exhibited also by the study of *Nimbkar et al.* (2006).

Effect on the Syrup pH. The largest drop in pH was observed in the syrup samples stored at 40°C and then

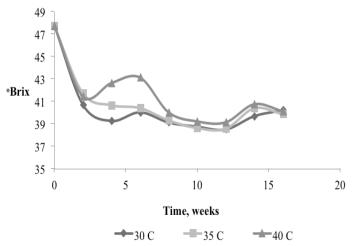


Figure 7. Mean Brix profiles of the sweet sorghum syrup samples stored in HDPE containers at 30, 35 and 40°C carbon steel (CS) and HDPE containers at 40°C.

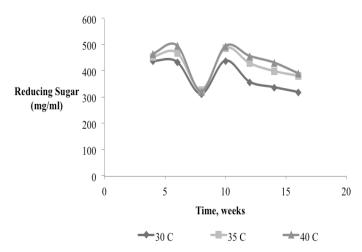


Figure 8. Average reducing sugar concentration profiles of sweet sorghum syrup samples stored in HDPE containers at 30, 35 and 40°C.

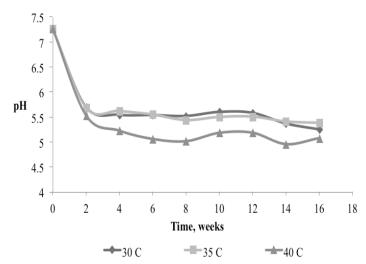


Figure 9. Average pH profiles of sweet sorghum syrup samples stored in HDPE containers at 30, 35 and 40°C.

those at 35°C (**Figure 9**). This result conformed with the study of *Lee* (2001) and *Wu et al.* (2010). Less reduction in the amount of reducing sugars and dissolved solids were observed over the specified period of time. This may be due to the activity of secreting organic acids which was much favored by the spoilage microorganisms than further degrading the sugars still present in the syrup.

#### CONCLUSION AND RECOMMENDATIONS

The storage stability of sweet sorghum (SPV 422) syrup in terms of Brix, reducing sugar concentration and pH was tested. The effects of sorghum syrup's initial Brix, containers' material of construction and storage temperature on selected physico-chemical properties were determined with the use of a temperature-controlled incubator for four months. Sampling and analyses of total soluble solids, reducing sugars and pH were conducted every two weeks. The parameters (i.e., initial Brix, container's material, incubation temperature) and their interaction had significant effects on all response variables (i.e., Brix upon storage, reducing sugar content, medium pH).

Syrup samples with the lowest initial Brix concentration had the highest degree of reduction in the amount of total soluble solids and reducing sugars. On the effect on pH, favorable initial Brix content was observed at 41°. For the type of storage container, it was found that HDPE is a better a material than carbon steel in prolonging the shelf life of the syrup samples. A significantly lesser degree of deterioration in Brix and reducing sugars was obtained by using HDPE. Conversely, a larger drop in pH was observed in syrup samples stored in HDPE than those in CS containers. In this study, HDPE was recommended for use rather than CS.

On the experiment on the effect of incubation temperature, the results on pH, Brix and reducing sugar analyses were compared. Among the tested temperatures, 40°C was considered to be the most ideal.

This storage stability test was limited to monitoring the syrup pH, total soluble solids (Brix) and reducing sugar concentration. Quantitative analysis of organic acids and microbial count in the samples were not conducted. Hence, the specific cause of sugar deterioration or of the syrup's storage instability was not clearly identified. Furthermore, it is recommended to investigate the influence of the sweet sorghum variety on the syrup stability. Exploring the use of chemical and/or biochemical agents to inhibit the growth of spoilage microorganisms may also be done.

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