Seventh framework programme
Food, Agriculture and Fisheries, and Biotechnology

Specific International Co-operation Actions
Small or medium scale focused research project

Sweet Fuel

Sweet Sorghum
an alternative energy Crop

Grant Agreement n° 227422

Deliverable 5.4:

Definition of a logistic harvest and raw material handling improving storability of sweet sorghum

Composition of the consortium

CIRAD
ICRISAT
EMBRAPA
KWS
IFEU
UniBO
UCSC
ARC-GCI
UANL
WIP

Definition of a logistic harvest and raw material handling improving torability of sweet sorghum

Authors: Walter Zegada-Lizarazu and Andrea Monti

1. Introduction

Nowadays one of the main bottleneck for sweet sorghum development is the lack of effective harvesting and storage techniques. The appropriate definition of such techniques has the potential to ensure economic benefits to sweet sorghum farmers and optimize ethanol production, especially in European temperate climates where sweet sorghum is a relatively new crop. Several authors, mainly in tropical and subtropical climates, found that sugar concentration and ^{o}Brix increase from flowering to ripening (Broadhead 1969; 1972; Tsuchihashi and Goto 2004), and therefore the harvest window is restricted to a relatively short period, becoming a determinant factor for projecting the dimension and operation times (e.g. seasonal or continuous) of a processing plant. In temperate climates, however, the optimal harvest time could be even shorter due to the limited effective growing season. Besides that, the harvest of un-defoliated stems may decrease the amount and quality of the extracted juice. Therefore, stripping the leaves in the field may not only improve the juice fermentation characteristics, but also contribute to the maintenance or increase of the soil fertility. Then, harvest time and method are important aspects that need to be considered for defining adequate sweet sorghum management and logistics in new environments such as those of temperate climates.

Typically, at harvest sweet sorghum has a high moisture content (70-80%), which leads to a rapid deterioration of the harvested material unless specific processes would allow stabilizing the biomass. Sugar deterioration, due to increased activity of contaminating bacteria and pH decrease, starts immediately after harvest if ambient temperature is above 15 ^{o}C (Bludau 1992; Wu et al., 2010). Whole-stem harvested material shows retarded signs of deterioration (about one week), while at the same time billet chopped stalks can lose about 50% of fermentable sugars (Eiland et al. 1983). In any case, the limited storability and fast decay of the harvested material and sugars either as whole or chopped stems, and therefore the limited time for its processing, are factors that can severely compromise the sweet sorghum production.

On-farm processing technologies for initial distillation with low-cost equipment and without any pretreatment or temperature control have been proposed as a promising storage technique to overcome the above mentioned concerns. Kunduyana et al. (2010), for example, found that it is possible to store sweet sorghum juice directly in the field upon its fermentation by yeasts. But, due to the usual large amounts of fructose left by the wide performance range of yeast cultures after the fermentation process has finished (Day and Sarkar, 1982; De Mancilha et al., 1984; Wu et al., 2010), spoilage losses could be large,

especially if the finished beer is stored for long terms under farmer's conditions. Therefore, the knowledge on preservation and storage of un-distilled sweet sorghum juice under low-resource settings and small-scale farmer's conditions is still in its infancy.

Ensiling and inoculating the residual bagasse, after juice extraction and fermentation, with a mixture of lactobacillus bacteria could be a valid strategy for an integrated farm-based harvest and storage management of sweet sorghum. However, little information is available about the influence of adding inoculants to the ensiled material on its conservation and methane production potential. The conservation of the ensiled sweet sorghum bagasse in the absence of oxygen can substantially limit the consumption of fermentable sugars and therefore maintain its energy value (Muck, 2010). Moreover, the inoculated lactobacillus bacteria (e.g. *Lactobacillus buchneri* and *Lactobacillus casei*) may enhance the hydrolysis of the lignoclellulosic material, and therefore facilitate the anaerobic bacteria digestion/fermentation process in order to produce methane. This integrated approach may bring about many advantages, from greater efficiency in the use of reaming sugars in the bagasse to maximizing the energy yield of sweet sorghum. Therefore, development of efficient harvesting techniques and methodologies that that would allow to harvest and store directly within small farmer's installations sweet sorghum juice as un-distilled ethanol and the remaining bagasse as ensiled material for further processing are needed.

2. An integrated approach to evaluate on-farm harvesting and storage techniques

The optimization of harvest methods and storage techniques under small farmers' conditions was evaluated in a series of trials using a commercial sweet sorghum variety (Sucro 506; Fig. 1).

The schematic setup of the different trails carried is shown in Fig. 2. In brief, the best harvest time for maximizing ethanol production as well as maximizing the accumulation of structural carbons was determined in combination with the evaluation of the fermentation capacity of two types of yeast cultures (beer and wine type) at two concentrations in Exp. 1.

In a follow-up trial, two harvest methods (defoliated and non-defoliated stalks) in combination with a mixture of different proportions of other two types of yeast (glucophilic and fructophilic) were tested. In addition the un-distilled ethanol obtained from the fermentation process was bottled and stored for six months (Exp. 2). In the last trial, the same glucophilic and fructophilic yeast types used in the previous one were evaluated, but in this case 100% concentration of each yeast type were inoculated. Besides that the storage period of the un-distilled ethanol was prolonged up to one year and the reaming bagasse was ensiled and inoculated with a mixture of lactobacillus (*Lactobacillus buchneri and L. casei*) bacteria to improve its conservation (Exp. 3).

Fig. 1. Harvesting and storage process used to evaluate different harvest methods and storage techniques under farmers conditions

In all trials the changes in soluble sugars and alcohols through the fermentation periods were determined by high-performance liquid chromatography (HPLC). In Exp. 3, the bagasse conservation quality was evaluated through the determination of volatile fatty acids, residual sugars, alcohols, and ammonia nitrogen.

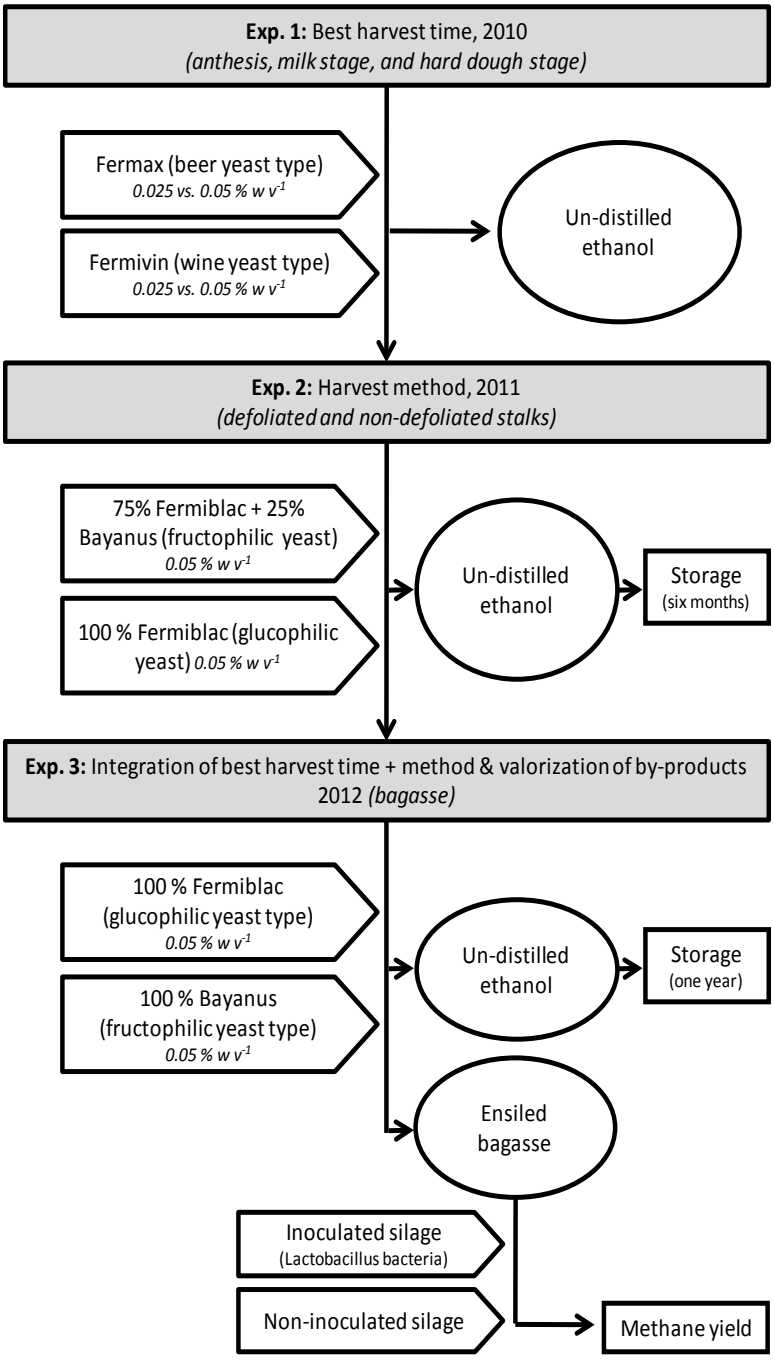

Fig. 2. Schematic representation of the integrated approach to harvest and storage sweet sorghum at farm scale

3. Harvesting times and methods, and in-field storage of un-distilled ethanol

The definition of appropriate harvesting methods and timing for maximizing ethanol production directly from sweet sorghum juice fermentation (1st generation biofuels) or from structural sugars (2nd generation biofuels) have the potential to ensure the economic benefits of sweet sorghum growth. Currently, however, the development of such

technologies under small farmers conditions in European temperate climates, where sweet sorghum is a relatively new crop, are limited. It is observed from figure 3 that the highest ethanol yields were obtained with fermax yeast at a concentration of 0.5 g l^{-1}; average yields were between 40 and 50 g ethanol per liter of juice. On the other hand, ethanol yields with fermivin yeast type were in the range of 14 and 38 g l^{-1} at the milk stage and hard dough stage, respectively.

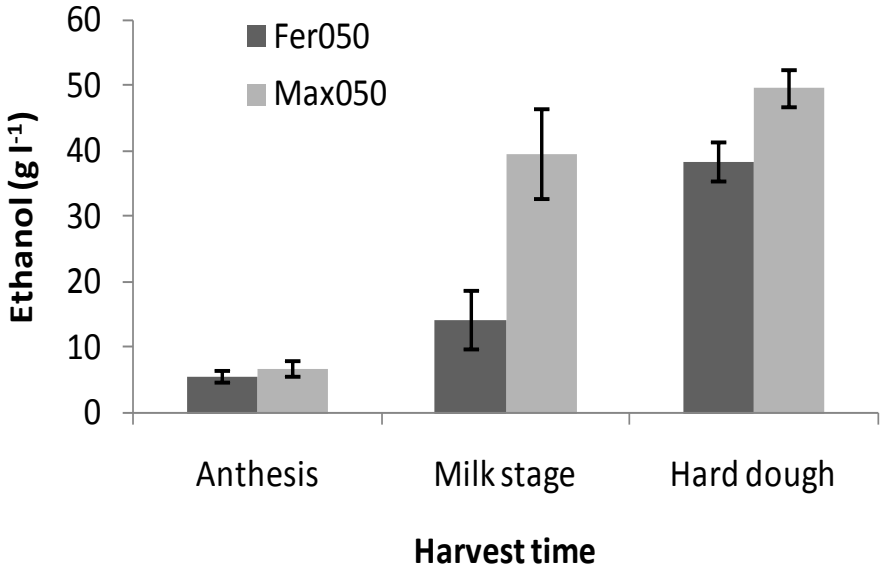

Fig. 3. Ethanol yield of sweet sorghum harvested at three growth stages and fermented with two yeast types (Fer; fermivin and Max; fermax).

Harvesting sweet sorghum at the anthesis stage significantly reduced the biomass yields and ^{o}Brix degrees, and therefore ethanol yield of both yeast cultures. Even though the determination of these growth stages is easy and well know to farmers, remains the fact that the best harvest window is still limited to only about 35 or 40 days, leaving short margin of time for its transportation and processing or other farming operations. Finding simple, cheap, and efficient ways to preprocess and/or store sweet sorghum under decentralized systems and farmer's conditions would secure feedstock supply to a centralized processing plant for its continue operation.

In addition it was found that leaf stripping at harvest and prior to juice extraction results in significantly higher ethanol yields than from un-stripped plants (Fig. 4). However, it remains to be determined if such higher ethanol yields could compensate the higher costs of defoliating the plants prior juice extraction. Moreover, the ethanol yields from the stripped

stalks' juice, inoculated either with fermiblanc (glucophilic yeast) or the mixture of glucophilic and a frutophilic (bayanus) yeast, produced in average about 2.5 times more ethanol than the unstripped stalks at the end of the fermentation process (Fig. 4).

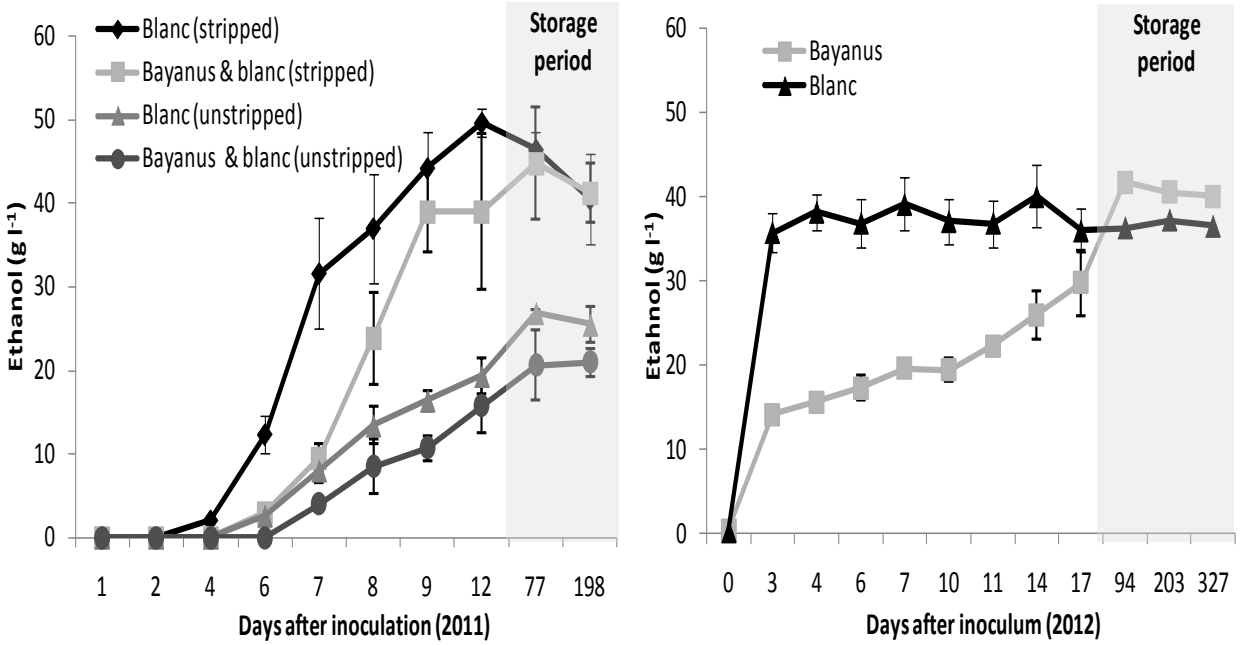

Fig. 4. Effect of harvest method and yeast type on the fermentation process of sweet sorghum juice and storage of un-distilled ethanol. Blanc; fermiblanc a glucophilic yeast type. Bayanus, a fructophilic yeast type.

Afterwards during the storage period the differences between the stripped and unstripped samples remained, but it is important to note that in comparison to the values reached at the end of fermentation process, the stripped samples showed a slight increase in ethanol yields with the bayanus-fermiblanc mixture. The unstripped samples also showed a slight increase in ethanol yields during the storage period. Complementary test carried out in Exp. 3 to compare the individual fermentation performance of bayanus and fermiblanc showed that the later one reached maximum ethanol yields faster than bayanus and that these yields were maintained throughout the storage period (about one year). On the other hand, bayanus increased significantly its ethanol yields during the storage period; at the end of the fermentation process bayanus produced about 30 g l^{-1} of ethanol, while during the storage period ethanol yields increased by about 37% (Fig. 4). The improved ethanol yields observed during the storage period when a frutophilic yeast strain was used could be related to the capacity that these yeasts have to restart stuck fermentation based on their affinity

for fructose (Tronchoni et al., 2009; Sütterlin, 2010). Even though both yeast types used here were able to perform well within a wide ambient temperature range, the fluctuating temperatures registered along the fermentation process may have been a factor for the contrasting performance. In any case, it was demonstrated that temperature control is not necessary for achieving acceptable fermentation rates under farmers' conditions if wide working range yeast types are used. Even though the ethanol yields obtained here were within the lower range of those reported in other studies that were carried out under controlled and non-controlled environmental conditions (Lui, et al. 1984; Kundiyana, et al. 2010; Ratnavathi, et al. 2010; Jia et al. 2013), the methodology employed here is susceptible to further improvements, especially if the triggering of the yeast fermentation process under non sterilized conditions are accompanied by efficient juice extraction methods and the selection of synchronized maturity cultivars with high ^{o}Brix.

4. Bagasse storage and conservation

Ensiling the bagasse with and without the presence of a mixture of *Lactobacillus buchneri* and *Lactobacillus casei* preserved the original structural and non-structural sugars (Fig. 5). It is well known that silage conservation is closely related to the development of anaerobic conditions and growth of lactic acid bacteria which results in an increased production of volatile compounds and therefore on improved storability (McDonald et al., 1991; Tabacco et al., 2011). In addition to that in our study it was demonstrated that by inoculating the ensiled bagasse with a mixture of lactic acid bacteria (*Lactobacillus buchneri* and *Lactobacillus casei*) it was possible to increase the production of volatile compounds such as lactic acid, acetic acid, and propionic acid (Fig. 5), which are the main acids that inhibit biomass degradation and prevent yeasts fermentation (Muck, 2010). Similar results were obtained in commercial maize silos (Tabacco et al., 2011) and, grass and small grains silages (Kleinschmit and Kung, 2006). As shown by others, *L. buchneri* has the ability to ferment lactic acid into acetic acid and therefore further improve the aerobic stability of the silage by inhibiting yeast and mold production (Muck, 2010). Besides that, ensiling prevented butyric fermentation as indicated by the minimal levels of butyric acid present in the silages (Fig. 5). Butyric acid fermentation is an indication of one of the most wasteful fermentation process that occurs in silages (Muck, 2010; Pyś et al. 2010). In our case, however, butyric acid was almost inexistent and dry matter losses of the inoculated bagasse were small (Fig. 5).

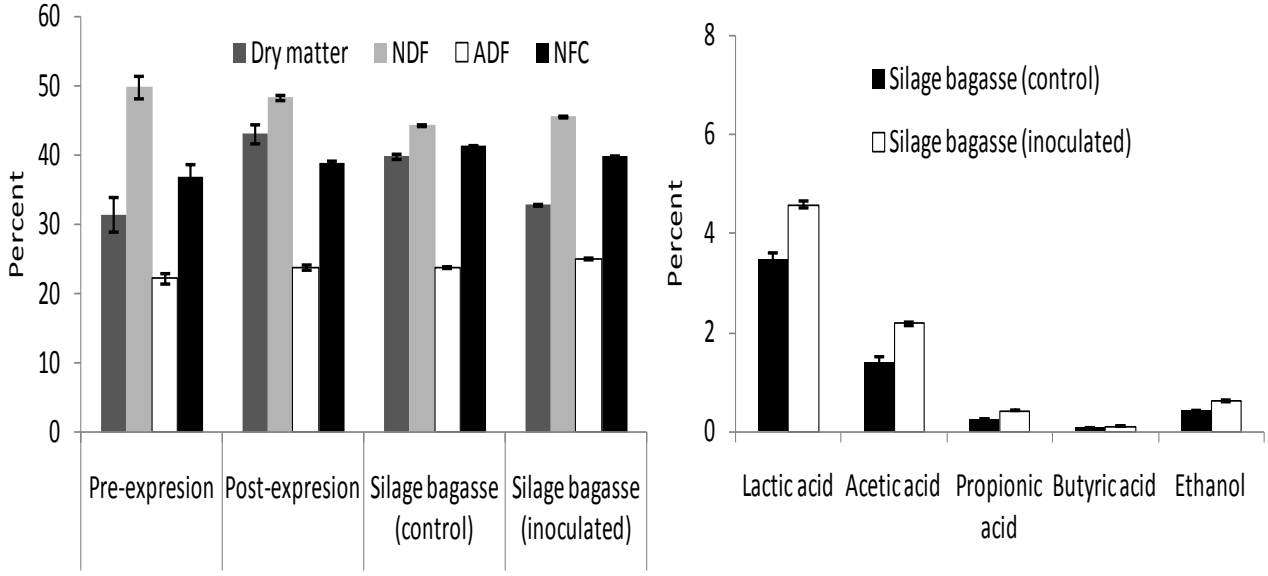

Fig. 5. Storage degradability of dry matter and structural and non-structural sugars, and preservative effects of Lactobacillus bacteria on ensiled sorghum bagasse

Such small dry matter losses were closely related to the enhanced production of acetic acid as it was well established to occur by the meta-analysis of the effects of *L. buchneri* on silages (Kleinschmit and Kung, 2006). Moreover degradation of structural and non-structural sugars after 60 days of ensiling were insignificant (Fig. 5), suggesting that alcoholic and bacterial fermentation was almost completely inhibited. The typical aerobic stability effects on silages by *L. buchneri* have been reported to occur from 45 to 60 days onwards after inoculation (Muck, 2010). Therefore, the improved storability of the inoculated bagasse may allow farmers to more precisely program the storage periods and optimize its utilization as bioenergy, chemical or any other feedstock with industrial application. In that way farmers could put on market the product on the most economically beneficial period with sustained energetic values.

5. Conclusions

It was demonstrated that harvesting around the hard dough stage and defoliating the plants before juice extraction result in higher ethanol yields. Moreover, the use of fructophilic yeast types allows to maximize un-distilled ethanol yields and store it for about one year without

spoilage damages. And the energetic and conservation properties of ensiled bagasse can be improved if inoculated with a mixture of lactobacillous bacteria.

6. References

Bludau, D A (1990) Harvest and storage of sweet sorghum In: 5th E.C. Conference biomass for energy and industry. Volume 1 Policy, Environment, Production and Harvesting. Lisbon, Portugal, 9-13 October 1989, pp.1260-1266.

Broadhead DM (1969) Sugar production from sweet sorghum as affected by planting date, after-ripe harvesting, and storage. Agron J 61:811-812.

Broadhead DM (1972) Effect of planting date and maturity on juice quality of Rio sweet sorghum. Agron J 61:811-812.

Day DF, Sarkar D (1982) Fuel alcohol from sweet sorghum:microbial aspects. Dev. Ind. Microbiol. 23: 361-366.

De Mancilha IM, Pearson AM, Waller J, Hogaboam GJ (1984) Increasing alcohol yield by selected yeast fermentation of sweet sorghum. I. Evaluation of yeast strains for ethanol production. Biotechnol. Bioeng. 26:632-634.

Eiland BR, Clayton JE, Bryan WL (1983) Losses of fermentable sugars in sweet sorghum during storage. Transactions of the ASAE; 1983, pp.1596-1600

Jia F, Chawhuaymak J, Riley MR, Zimmt W, Ogden KL (2013) Efficient extraction method to collect sugar from sweet sorghum. Journal of Biological Engineering 7:1-8

Kleinschmit DH and Kung Jr. L. (2006) A Meta-Analysis of the Effects of Lactobacillus buchneri on the Fermentation and Aerobic Stability of Corn and Grass and Small-Grain Silages. J. Dairy Sci. 89:4005 - 4013

Kundiyana DK, Bellmer DD, Huhnke RL, Wilkins MR, Claypool PL (2010) Influence of temperature, pH and yeast on in-field production of ethanol from unsterilized sweet sorghum juice. Biomass and Bioenergy 34:1481-1486

McDonald P, Henderson AR, Heron SJE (1991) The Biochemistry of Silage. 2nd ed. Chalcombe Publications, Marlow, UK.

Muck RE (2010) Silage microbiology and its control through additives. R. Bras. Zootec. 39:183-191.

Pyś JB, Karpowicz A, Szałata A (2010) The effect of harvest date and additives on chemical composition and aerobic stability of sorghum silage. Slovak J. Anim. Sci. 43:187-194.

Ratnavathi CV, Suresh K, Vijay Kumar BS, Pallavi M, Komala VV, Seetharama N (2010) Study on genotypic variation for ethanol production from sweet sorghum juice. biomass and bioenergy 34:947- 952

Sütterlin K. A. (2010) Fructophilic yeasts to cure stuck fermentations in alcoholic beverages. PhD Thesis. Stellenbosch University. Institute of Wine Biotechnology, Faculty of AgriSciences

Tabacco E, Piano S, Revello-Chion A, Borreani G (2011) Effect of Lactobacillus buchneri LN4637 and Lactobacillus buchneri LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. J. Dairy Sci. 94:5589–5598

Tronchoni J, Gamero A, Arroyo-López FN, Barrio E, Querol A (2009) Differences in the glucose and fructose consumption profiles in diverse Saccharomyces wine species and their hybrids during grape juice fermentation. International Journal of Food Microbiology 134: 237–243

Tsuchihashi N, Goto Y (2004) Cultivation of sweet sorghum (*Sorghum bicolor* (L.) Moench) and determination of its harvest time to make use as the raw material for fermentation, practiced during rainy season in dry land of Indonesia. Plant Prod Sci 7:442-448.

Wu X, Staggenborg S, Propheter JL, Rooney WL, Yub J, Wang D (2010) Features of sweet sorghum juice and their performance in ethanol fermentation. Industrial Crops and Products 31:164-170