

# Sugarcane to Bioethanol - Feedstock Management

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## ABSTRACT

*Taiwan lacks fossil fuel resources and thus highly depends on imported oil. Ethanol can be used as a substitute for gasoline as well as an additive to improve gasoline combustion quality. Bioethanol is commercially produced by using starch, high sugar containing crops, or cellulosic material in much smaller scale. Among all possible feedstocks, sugarcane is considered as a superior choice in tropical to subtropical areas than others. However, the success of fuel ethanol production relies heavily on the stable supply of economic feedstocks.*

*To evaluate the feasibility of using sugar cane as the feedstock for fuel ethanol production in Taiwan, a study was carried out to investigate the capability of ethanol production per hectare of farm. From 2009 to 2011, 8 varieties of sugar canes with various maturing rates were planted on farms, chosen according to soil types. Sugar canes were sampled periodically and analyzed for total biomass, sugar contents, and sugar compositions. Using cane juice as the single substrate for *Saccharomyces cerevisiae*, ethanol production yields for sugar canes were investigated. Systematical arrangements of planting and harvesting produced various samples enabled us to look into the effects of various combinations of conditions, such as cane varieties, age of canes, time of planting, etc. Ethanol production potential per hectare of land was estimated by combining biomass produced per hectare and the ethanol yield per unit mass of sugar cane. In addition to the supply of the feedstock, the storage duration of post-harvest sugar cane might be an issue for bioethanol production.*

*Samples of various canes from stocks with staling times ranged from 0-9 days were investigated. The temperature, during the period of the experiment, was found to fluctuate from 25 °C at night to 33 °C at high noon. The weight and sucrose content of cane samples decreased rapidly and the inverted sugar increased after five days. Interestingly, the ethanol yield per unit mass of cane juice did not seem to be significantly affected by the staling time.*

*This research has generated a significant amount of information. A database has been composed accordingly, which can be used to estimate ethanol yields using basic cane properties such as varieties, age of cane, as well as the component and quantity of total sugar. This would be a helpful tool in feedstock management for a cane ethanol producer.*

Keywords: Sugar cane, Bioethanol, Renewable Energy

## INTRODUCTION

A persistent challenge that confronts Taiwan is the reliance on imported energy. Among the various forms of energy demands, liquid fuels, which mostly are produced from fossil oil, are among the immediate needs in daily life. The stability of oil supply is closely monitored by the government due to its potential impact up to the state level. The volatility of oil price also constantly tests Taiwan where manufacturing and transportation play pivotal roles in economic activities. Another problem accompanying massive demand on fossil fuels is the inevitably high-

volume greenhouse gas emissions. Thus, sustainable alternative energy has drawn much attention of Taiwan to partially tackle energy problem without adding any more burden to the environment.

Ethanol (or bioethanol) can be used as a substitute for gasoline as well as an additive that improves combustion quality of gasoline. Bioethanol is commercially produced from starch, high sugar content crops, or (on a much smaller scale) from cellulosic materials. Although sugarcane is considered to be a superior choice among all possible feedstocks in tropical to subtropical areas due to its high energy conversion efficiency (Farrell, 2006) and water efficiency (Gerbens-Leenes and Hoekstra , 2009), the sugarcane based bioethanol industry still faces some challenges. For example, the possible availability of a stable feedstock supply needs to be addressed before setting up a commercial scale sugarcane ethanol production site.

In Taiwan, a sugarcane harvesting season generally starts from November and lasts until March of next year. Traditionally, sugarcane can be planted during spring (spring planting, from January to March), autumn (autumn planting, from July to September), or use ratooning for a few harvest seasons. The growth period of sugarcane takes 12-18 months. Sugarcane planted in summer does not yield enough sucrose as a suitable feedstock for sugar mills before the harvest season of that year ends. However, its higher content of reducing sugars, such as glucose and fructose, can be transformed into ethanol by microorganisms. In order to evaluate the feasibility of using sugarcane as the year-round feedstock for bioethanol production, we constructed a sugarcane database that included the characteristics of sugarcane produced under various conditions.

## Materials and Methods

### Sugarcane varieties

Eight sugarcane varieties were used in this study, namely ROC 1, ROC5, ROC9, ROC10, ROC16, ROC24, ROC26, and F160. These cane varieties were developed at the Taiwan Sugar Research Institute specifically for sugar farms in Taiwan.

### Field experiment

Field experiments were carried out in Chiayi County, Taiwan (23.2°N latitude, 120.1°E longitude), where one of the major supplies of sugarcane comes from for sugar mills. Sugarcane was planted on 3 different sites located not far from each other. Each of the 3 sites has its own characteristic soil types of sandy loam, loam, or clay. During the period of this study, the associated monthly rainfall and the maximum and minimum temperature, for this area, are presented in Table 1. The 3 sites of geographically close were picked hoping to minimize the effects of climate factors.

### Biomass and sugar composition analysis

All samples were collected between 2009 and 2011 from crops planted in the month of April. Four stalks were randomly picked and harvested. The collected samples were pressed to obtain juice for further investigation.

### Juice analysis

Cane juice samples were analyzed for their sugar compositions using High-performance liquid chromatography (HPLC, Waters 2690 Separations module) equipped with an appropriate column (Waters Sugar Pak 1 cation exchanged resin-based column, 6.5x300 mm) and an RI detector (Waters 2410 Refractive index detector). The column was kept at 90°C during analyses. Mobile phase was 0.5g/L calcium EDTA aqueous solution with a flow rate of 0.6 mL/min. Juice samples were filtered to 0.45µm before HPLC analysis.

### Postharvest deterioration of cane

Cane samples of various varieties at the loam soil site were harvested on June 22nd 2011 and kept indoors. The maximum ambient temperature was approximately 33 °C during the experiment. Three cane stalks of each variety were randomly selected to collect juice at 1, 2, 5, 7 and 9 days. The juice was analyzed for sugar content and used for ethanol production tests.

### Ethanol Production

#### Microorganism

A strain of *Saccharomyces cerevisiae* (BCRC 920059) was used for ethanol production throughout this study. The strain was isolated from an ethanol production site of Taiwan Sugar Corporation and later used for commercial production of ethanol.

#### Seed preparation

Yeast cells were cultivated in a YM medium (Yeast Extract 0.3%, malt extract 0.3%, Peptone 0.5%, glucose 1%) in a 5L fermenter (Bioflo 3000 Batch/Continuous bioreactor, New Brunswick Scientific) at 30°C, with a stirring rate of 300 rpm. Aeration rate was controlled at 1vvm. After cultivating for 16 hours, the broth was centrifuged at 5,400 g for 1 min to harvest yeast cells. The harvested cells were then re-suspended in sterilized water to give a turbidity of 300±10 at 600nm.

#### Cane juice

Cane juice was collected from crushed and pressed sugar cane samples. Cane juice samples were concentrated using evaporation if its total sugar content was less than 12 %. No further treatment or fortification of other ingredients was applied to cane juice prior to fermentation.

#### Ethanol production

Seed solution (10mL) and sugarcane juice (90 mL) were mixed in a 250 mL flask. The fermentation was carried out at 30°C without shaking. Sucrose, glucose, fructose and ethanol contents of the tested samples were determined using HPLC as previously described.

## Results and Discussion

### Sugar composition analysis

Sucrose content is almost always considered as the most important factor to evaluate a sugarcane variety. For cane sugar industry, sugarcane needs 12-18 months to grow and accumulate enough sucrose for further processing. Sugarcane grows rapidly during summer as natural resources are plentiful, e.g. abundant rainfall and sufficient sunlight. The main product of photosynthesis, i.e. glucose, is used as the building block and energy sources to fuel cell growth, and consequently turned into biomass. As temperature gradually decreases after summer, sucrose synthesis seems to surpass cane growth and give rise to an increase in sucrose content in sugarcane. The temperature of 15°C was considered to be critical for cane growth (Hsia, 1972).

Total sugar content and sugar composition were analyzed for samples of different cane ages, soil types, and varieties (Fig 1-3). The contents of reducing sugars, i.e. glucose and fructose, were observed to decrease during the period from November to April. Sucrose was found to be the major sugar species present during the period. Sugar yields per hectare were estimated by combining the results of sugar content in biomass and harvested biomass per hectare (Table 2-4).

In a sugar mill, reducing sugar in cane juice does not contribute to the yield of crystal sugar. On the contrary, reducing sugars may cause troubles during processing. Thus, traditionally, sugarcane with high reducing sugar content would be regarded as unsuitable for sugar production. However, *S. cerevisiae* is normally capable of using a wide spectrum of sugar species and taking advantage of the additional sugar besides sucrose. Therefore, the details recorded, relating to composition and sugar contents, in all the cane species during non-traditional harvest times may help optimize land use with respect to ethanol feedstock production. For example, the two early mature varieties, i.e. ROC1 and ROC 16, may be harvested as early as 10 months after planting for ethanol production, even though they were not considered as good feedstocks for making crystal sugar at the same age.

### Ethanol production test

Ethanol production yield out of the total sugar in cane juice was investigated as described in Materials and Methods. The result was summarized in Table 5. All the data were analyzed by using one-way ANOVA (Figure 4). Cane juice from 6-15 months old sugarcane gave satisfied ethanol yield under the described test conditions. The result that juice from 6 or 9 months sugarcane gave yields comparable to 12 to 15 months samples indicates that young sugarcane may be used as ethanol production feedstock with little upset in ethanol yield if necessary. Among the teste varieties, we have observed that ROC 5 and ROC 26 gave a lower ethanol yield than other varieties. A nutrient imbalance for yeast fermentation was suspected for the two varieties. Further investigations are needed to clarify this mystery.

### The characteristics of postharvest cane

Weng et al. summarized the reasons leading to the deterioration of postharvest sugarcane as the following: 1. the activity of invertase in the postharvest sugarcane; 2. consumptions by microorganisms. Also, the deterioration rate of harvested cane might be affected by cane varieties and age. In many cases, microbial activities result in acidic metabolites and thus change pH value. The pH value of juice from sugarcane samples going through various storage durations was measured. In Fig 5, it is shown that the pH value of cane juice decreased mildly after storage. Some juice samples could reach a lowest pH level of 4.85. Since *S. cerevisiae* can normally withstand a much more acidic environment, the pH change during postharvest storage is unlikely to be an inhibition factor if sugarcane is used as ethanol production feedstock. The rapid decrease of sucrose and considerable increase of glucose and fructose indicate that invertase was active during the period of observation (Figure 6-8). During the storage time, dehydration occurred due to the evaporation of water to the environment. Rate of dehydration and the sugar composition change were taken as the key factors of the evaluation of deterioration. The experimental result indicated that ROC16 and ROC 5 seem to deteriorate slower than other varieties. The ethanol yield, presented in Table 6 shows that the ethanol yield of tested varieties was not significantly affected by storage. This result shows that the “deterioration” of storage sugarcane in traditional sugar industry may not show if the same sugarcane is used for ethanol production since the total sugar did not decrease due to composition change.

## CONCLUSION

This research has gathered a significant amount of information, upon which a database has been compiled. Since ethanol yields of cane juice can be estimated using basic cane properties, such as cane variety, cane age, along with total sugar content and quality, the database can be used to estimate ethanol yields for commercial scale cane ethanol production with relatively complex sugarcane supplies as feedstocks. The database can be expanded to cover more parameters, such as climate factors, planting time etc. Furthermore, the possible scenarios of postharvest deterioration were discussed in this research. The result shows that ROC16 has high sugar content and a slow deterioration rate. ROC16 is, thus, recommended to be a good variety as the feedstock for ethanol production. The database should serve as a helpful tool in feedstock management for cane ethanol producers.

## REFERENCES

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Table 1. Monthly rainfall and temperature during the experiment

Month	Year	Rainfall(mm)	Maximum Temp °C	Minimum Temp °C
January	2009	0	27.9	4
February	"	3.5	34.7	13.6
March	"	72.5	30.8	10.6
April	"	59.5	31.9	13.6
May	"	5.5	34.4	17.6
June	"	301.0	36.2	20.6
July	"	134.5	36.6	22.9
August	"	655.0	37.1	24.8
September	"	40.5	36.0	24.6
October	"	8.5	35.2	17.1
November	"	4.0	31.5	15.8
December	"	13.0	29.8	7.6
January	2010	12.5	28.2	5.8
February	"	23.5	32.7	9.7
March	"	6.0	32.3	7.2
April	"	49.0	33.7	14.1
May	"	108.5	34.2	17.7
June	"	174.5	34.8	20.8
July	"	472.5	35.2	22.6
August	"	69.5	36.1	23.6
September	"	271.5	33.6	22.3
October	"	22.0	33.2	15.8
November	"	17.0	28.4	13.8
December	"	17.5	29.7	5.2

Table 2. Ethanol yield from cane juice (planted crop in April) at different cane age (6, 9, and 12 month)

Varieties	Soil type								
	<u>Loam</u>			<u>Sandy loam</u>			<u>Clay</u>		
	6	9	12	6	9	12	6	9	12
ROC1	86.0	94.4	89.1	91.4	90.7	92.8	89.4	88.3	78.6
ROC9	88.9	90.8	90.3	90.0	89.5	99.9	93.6	94.4	94.4
ROC10	85.0	93.5	75.3	89.4	91.1	99.9	95.8	91.6	98.1
ROC16	89.0	90.4	88.9	89.0	89.3	94.8	90.0	91.7	92.9
ROC24	94.4	83.1	86.0	95.8	87.3	99.9	91.1	86.8	84.8
ROC5	99.9	84.9	89.4	87.7	88.3	89.5	96.6	88.4	99.5
ROC26	84.8	78.0	79.2	91.2	89.6	88.6	91.6	86.4	85.2
F160	88.4	86.4	88.1	84.0	88.9	89.5	89.8	87.7	94.1

Table 3. Total sugar yields per hectare of different varieties of sugarcane in consecutive month during the investigation (loam)

Varieties	2009			2010				
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.
ROC1	9.7	10.2	12.0	12.1	8.7	12.0	13.3	13.1
ROC9	7.3	8.9	8.5	11.5	8.7	11.6	13.7	9.5
ROC10	8.9	11.5	11.8	13.7	14.5	10.9	15.4	14.5
ROC16	5.8	7.6	8.3	7.9	12.9	12.5	12.7	9.6
ROC24	6.8	9.2	6.3	7.9	7.1	7.9	7.0	7.7
ROC5	4.5	5.9	3.9	5.2	5.5	7.6	5.9	7.2
ROC26	3.5	2.9	3.3	7.1	4.9	5.5	4.7	5.9
F160	6.7	7.5	16.8	8.8	9.0	14.8	16.9	11.3

Table 4. Total sugar yields per hectare of different varieties of sugarcane in consecutive month during the investigation (clay)

Varieties	2009			2010				
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.
ROC1	4.5	5.9	4.3	5.3	7.1	9.3	9.6	8.1
ROC9	2.7	2.6	3.3	3.4	3.7	3.1	3.6	2.8
ROC10	2.2	4.1	3.6	3.6	2.8	3.6	3.2	4.5
ROC16	4.2	4.7	3.8	4.7	8.3	4.6	4.3	8.9
ROC24	4.1	6.5	2.1	4.5	4.1	6.9	5.5	6.2
ROC5	1.6	2.0	3.5	1.6	2.1	1.7	2.2	2.5
ROC26	0.9	1.0	2.7	0.9	0.7	1.2	1.0	0.9
F160	4.7	5.7	1.3	5.7	5.6	7.0	7.7	7.8

Table 5. Total sugar yields per hectare of different varieties of sugarcane in consecutive month during the investigation (sandy loam)

Varieties	2009				2010			
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.
ROC1	10.6	10.2	12.0	20.1	12.5	16.4	19.8	15.0
ROC9	8.4	8.9	8.5	16.4	11.8	9.2	12.1	8.0
ROC10	10.6	11.5	11.8	14.4	12.5	19.7	19.1	13.0
ROC16	9.2	7.6	8.3	19.6	12.9	13.4	18.1	12.3
ROC24	7.8	9.2	6.3	11.7	7.9	10.7	11.4	11.1
ROC5	8.1	5.9	3.9	11.8	9.0	11.2	12.7	10.2
ROC26	7.8	2.9	3.3	17.1	13.1	14.8	14.6	15.9
F160	9.3	7.5	16.8	19.0	16.8	18.1	18.4	11.7

Table 6. The ethanol yield of postharvest sugarcane

Varieties	Ethanol yield (%)			
	<u>1 day</u>	<u>5 days</u>	<u>7 days</u>	<u>9 days</u>
ROC1	85.6±1.6	83.6±1.2	87.3±2.9	76.9±9.4
ROC16	88.3±1.2	87.9±1.9	88.7±1.4	86.6±3.0
ROC24	89.6±2.0	87.7±2.0	87.7±1.6	86.3±1.9
ROC5	89.3±2.0	87.0±1.9	85.0±1.6	87.6±2.9
F160	89.4±2.1	90.6±2.9	99.0±0.3	87.4±4.1

Ethanol yield %= % ethanol /%theoretical ethanol X 100%



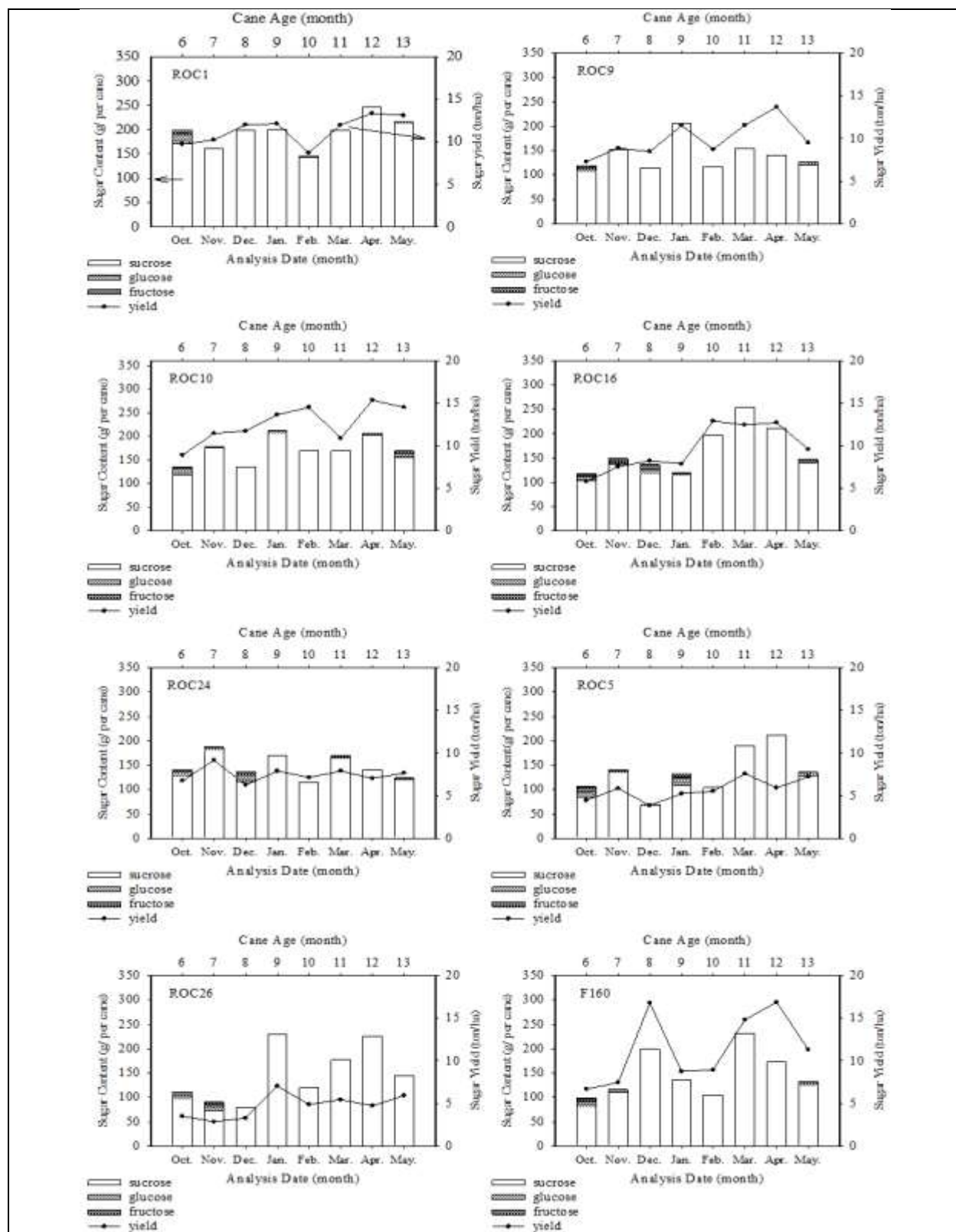


Fig. 1. Compositions of major sugar species and total sugar yields of spring crop samples on loam in consecutive months during the investigation.

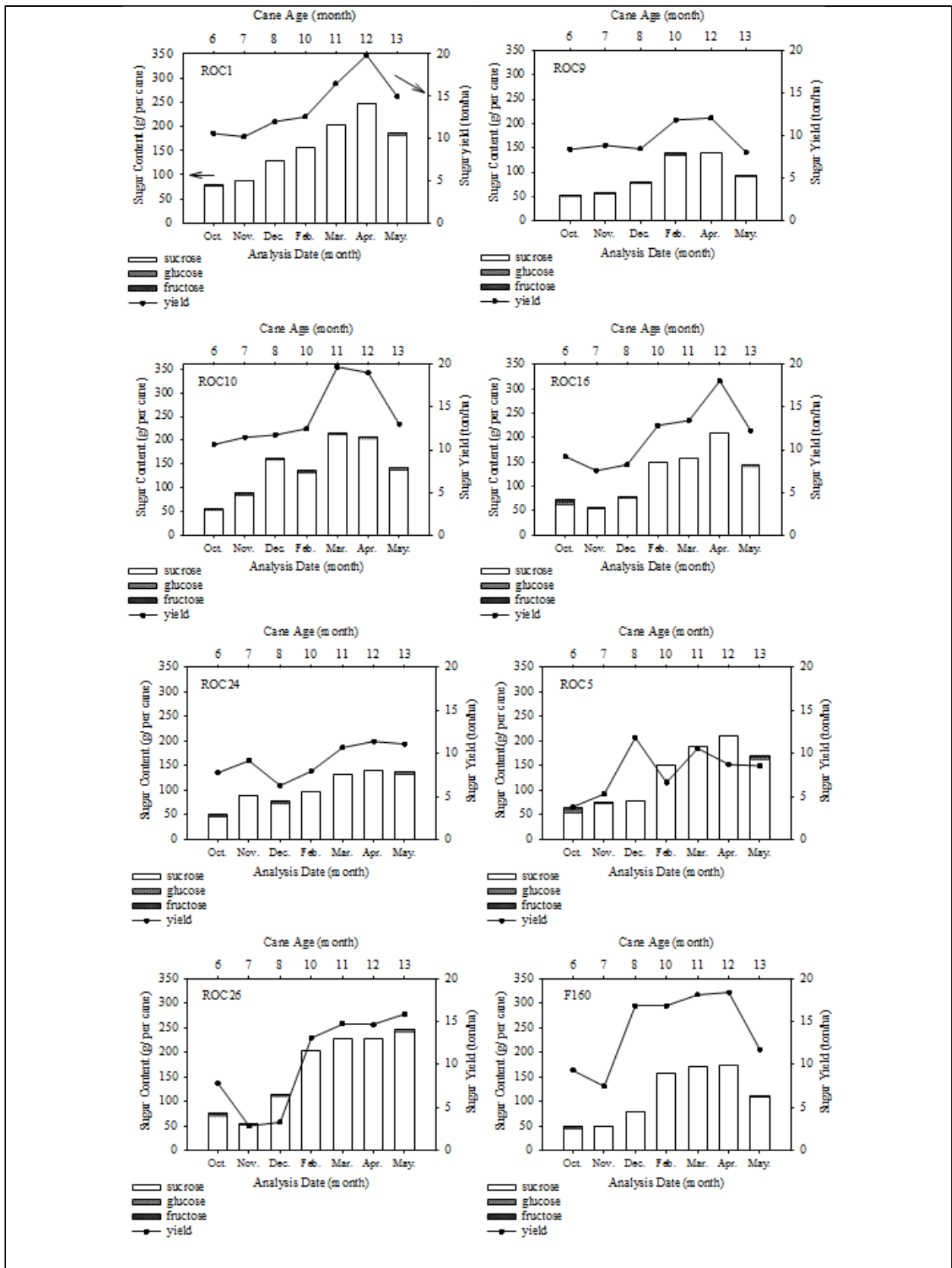


Fig. 2. Compositions of major sugar species and total sugar yields of spring crop samples on sandy loam in consecutive months during the investigation.

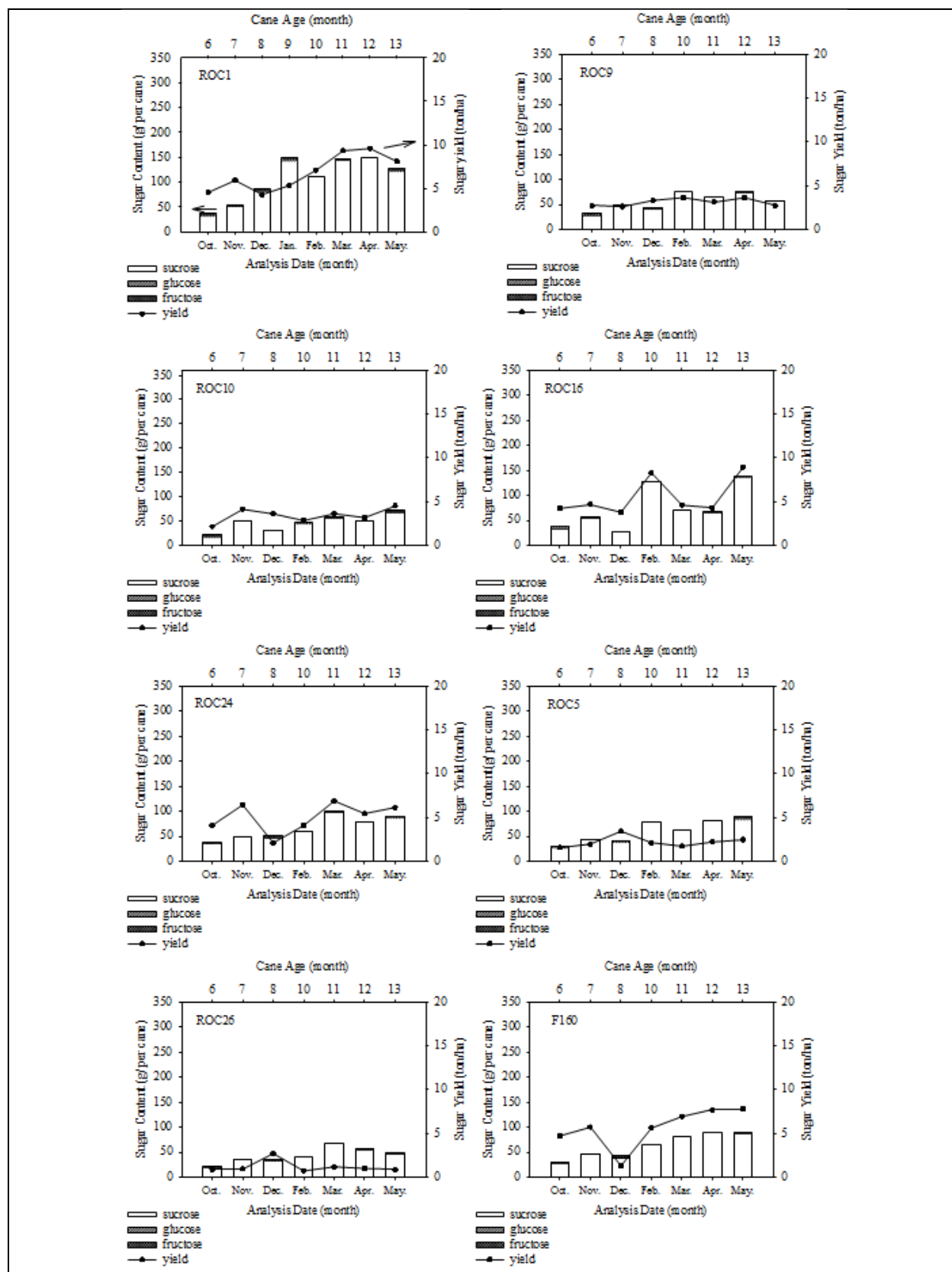


Fig. 3. Compositions of major sugar species and total sugar yields of spring crop samples on clay loam in consecutive months during the investigation.

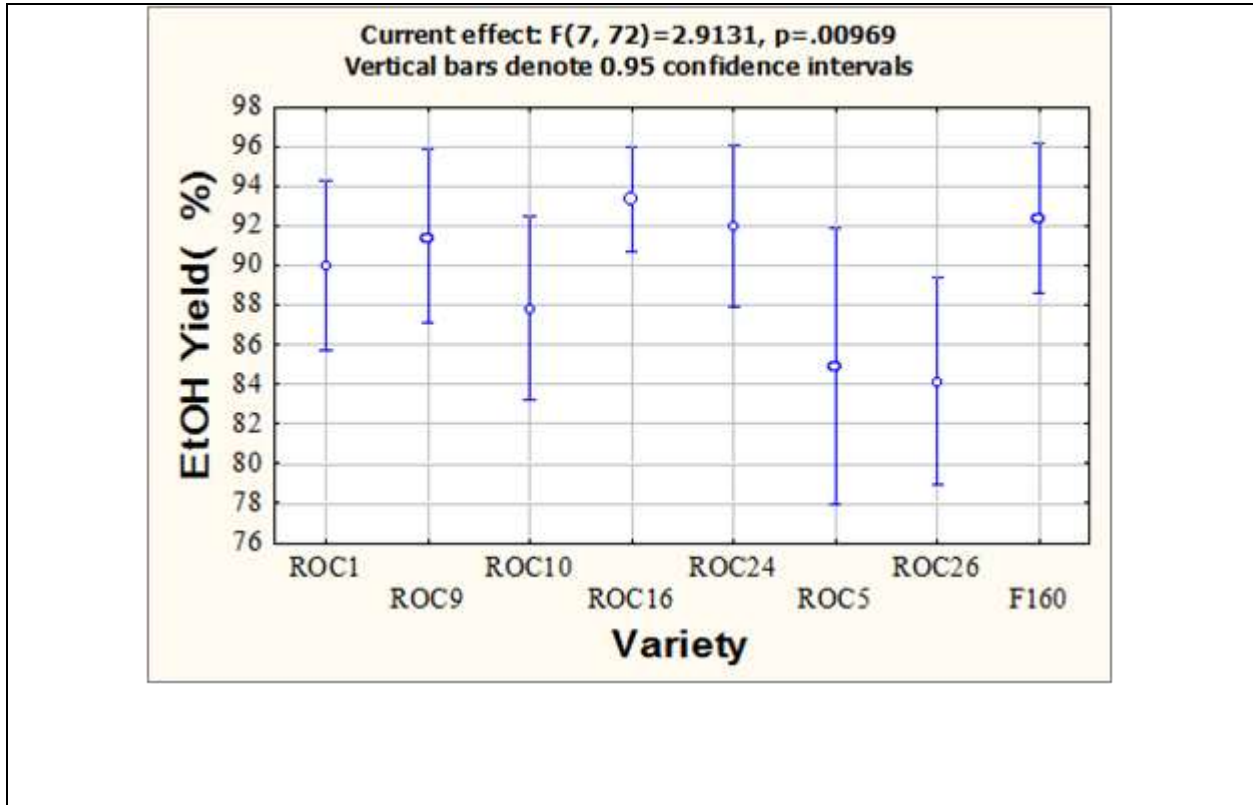


Fig. 4. The evaluation of the ethanol yield of cane juice from different varieties. (Planted on loam, year-round data)

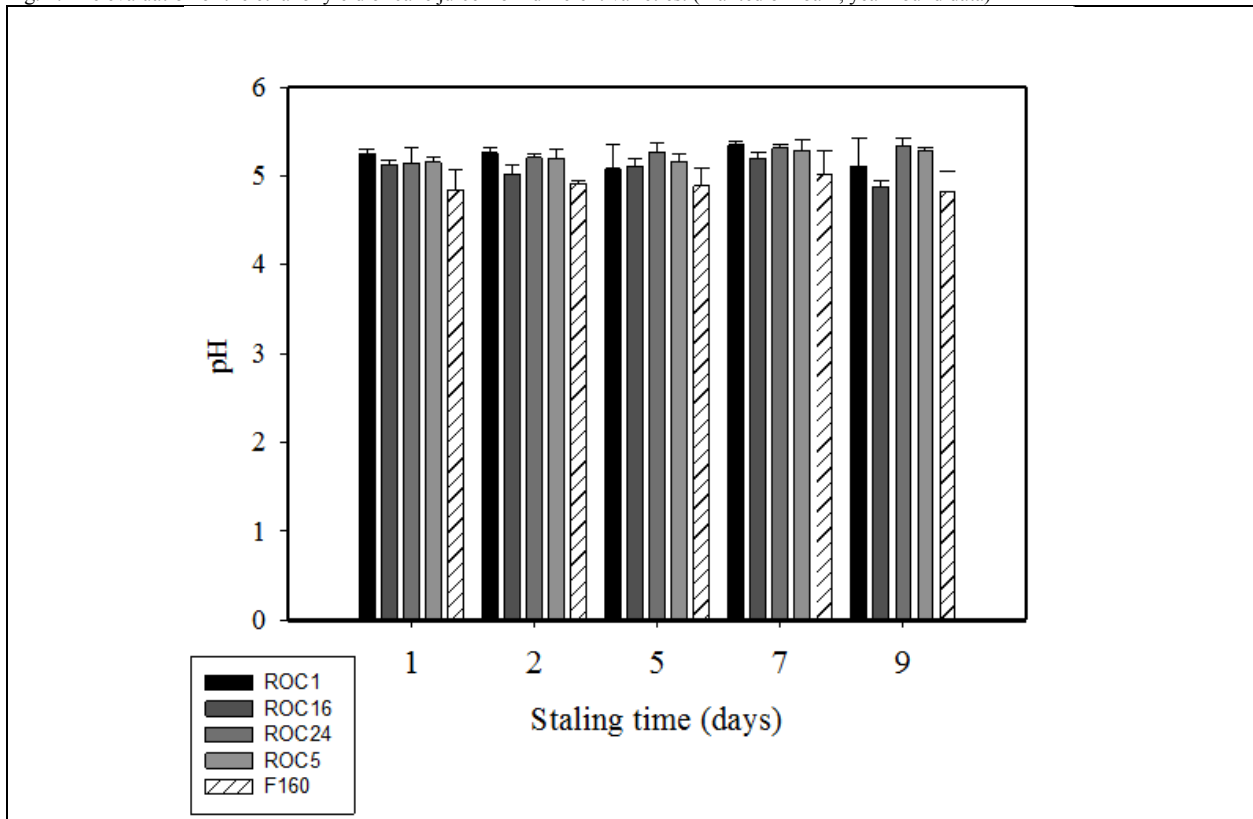


Fig. 5. The pH value of juice at different staling time.

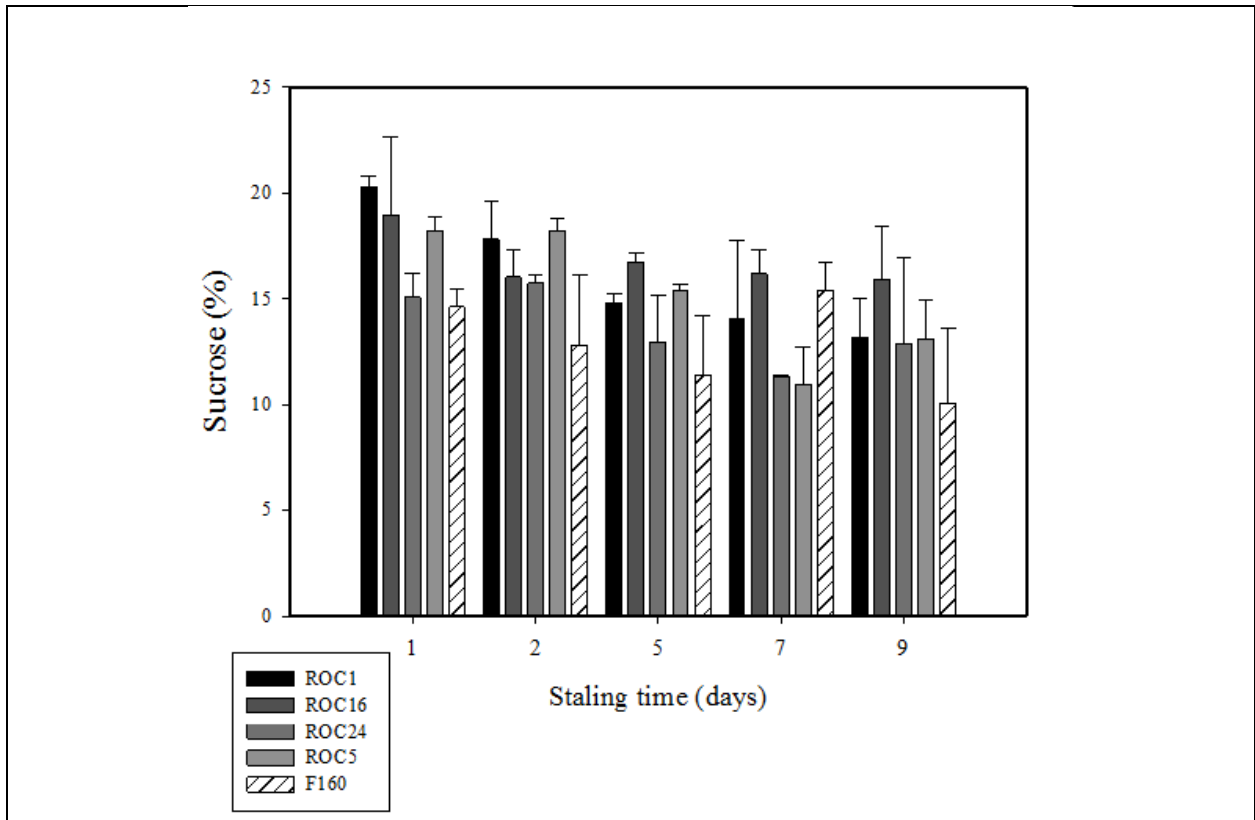


Fig. 6. The sucrose content changes in juice at different staling time

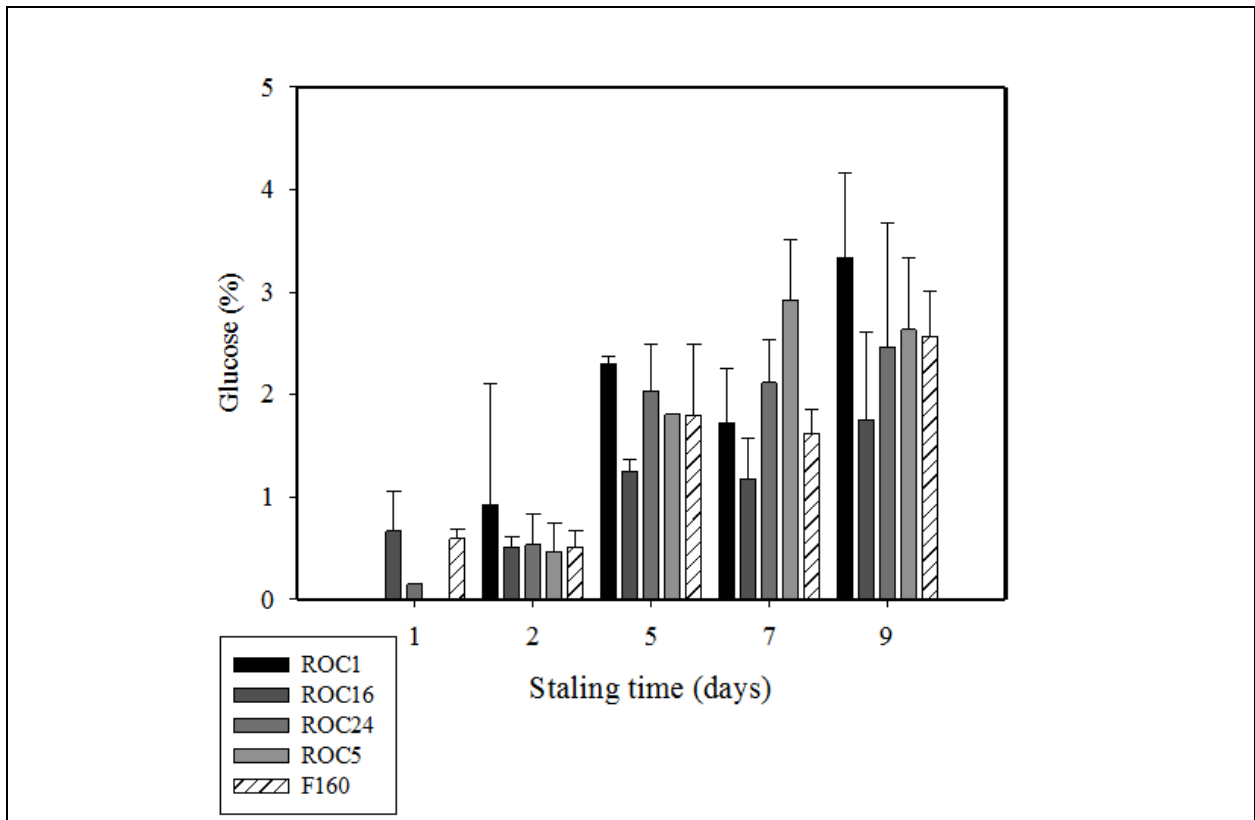


Fig. 7. The glucose content changes in juice at different staling time

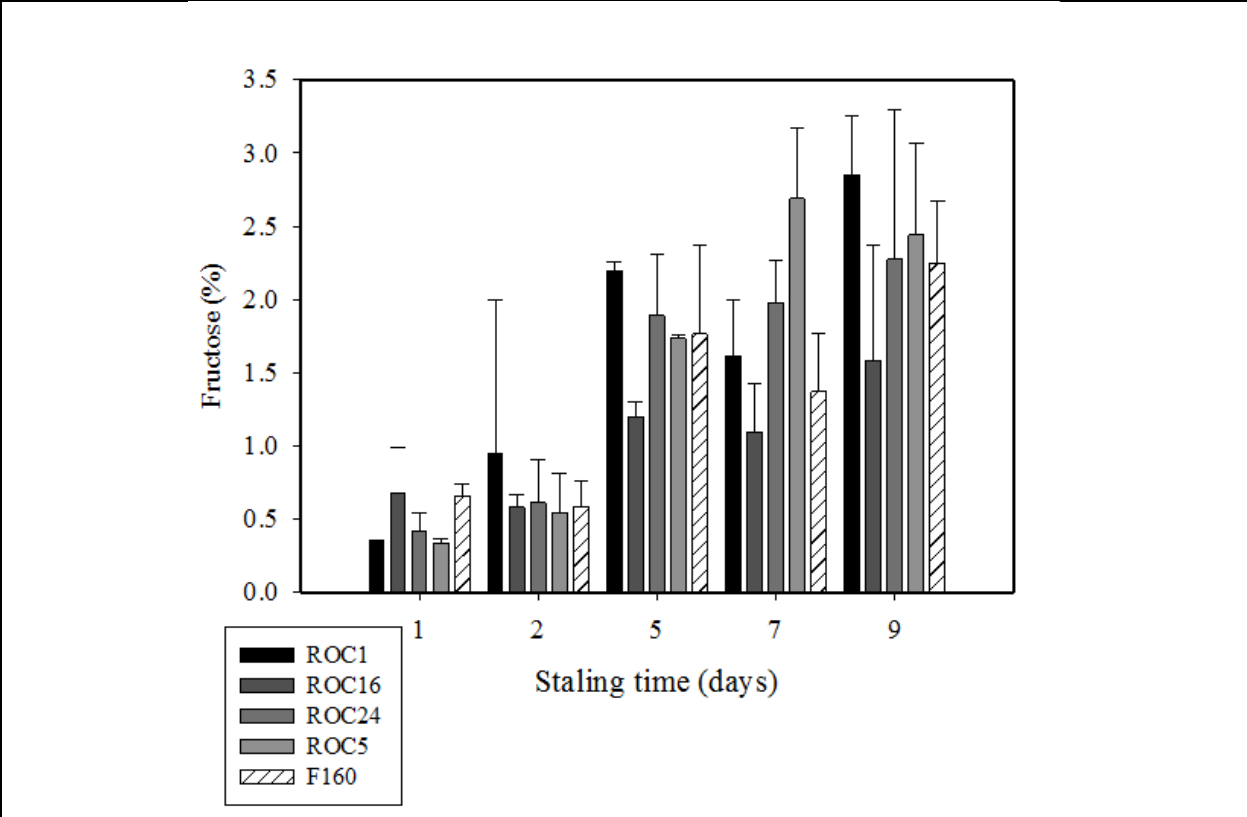


Fig. 8. The fructose content changes in juice at different staling time