EFFECT OF DELAYED PROCESSING ON ALCOHOL YIELD OF FRESH ROOT CROPS

Edgardo E. Tulin and Emma S. Data

Science Research Specialist and Associate Professor, Philippine Root Crop Research and Training Center, Visayas State College of Agriculture, ViSCA, Baybay, Leyte, Philippines.

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ABSTRACT

Delayed processing significantly affected the starch and total sugar contents of cassava and sweet potato roots. However, there is no apparent trend to show the relationship between delayed processing and starch or sugar content of the roots.

Alcohol production was significantly influenced by late processing in sweet potato but not in cassava. The highest alcohol content was obtained from sweet potato roots processed 2 days after harvest and fermented for 96 hours. In cassava, the amount of alcohol produced did not significantly vary even if the roots processed were fresh or stored up to 5 days under ambient conditions.

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KEY WORDS: Delayed processing. Cassava. Sweet potato. Alcohol production. Fermentation. Alcohol yield. Total sugar. Starch.

INTRODUCTION

Root crops, particularly cassava and sweet potato, are high in carbohydrate content and hence, can be good substrates for alcohol production. Fresh cassava roots contain about 30% fermentable substances (Grace, 1977). On dry weight basis, sweet potato roots contain about 1.5-2.5% sugar and 60-70% starch. Depending on the variety and method of manufacture, Grace (1977) and Amerine et al. (1972) cited that absolute alcohol yield ranges from 70-100 liters/ton of cassava and sweet potato roots.

Matsuoka et al. (1982) studied the alcoholic fermentation of raw sweet potato to alcohol through a one-step process combining liquefaction, saccharification and fermentation. The enzyme used for saccharification was glucoamylase of *Rhizopus* sp. and the yield of ethanol in the fermentation broth reached 94%.

Many studies on alcohol production utilizing tropical root crops were concentrated mainly on using the newly harvested roots as raw material (Grace, 1977; Matsuoka et al., 1982). However in actual situation, freshly harvested roots could not always be available particularly if the area of production is far from the processing plant. In large continuous operations, it may be necessary to procure roots from distant places in order to cope with the demand for fresh roots. It may take 2 or more days to transport the roots from the place of production to the processing plant or even longer if transportation is a problem. Thus, delays in the processing of fresh root crops for alcohol production are usually inevitable. Moreover, although it is possible to build alcohol processing plants in the primary root crop growing areas of the country, this is not practical because the cost of the plant is extremely prohibitive.

Delayed processing of cassava and sweet potato roots results in two major physiological disorders, i.e., vascular streaking in cassava and shrivelling in sweet potato. These disorders seriously affect the food value of the roots. However, it is not known whether or not they could also affect the roots' processing potential for alcohol production.

This paper presents the effect of delayed processing of cassava and sweet potato roots on alcohol yield, and on starch and total sugar contents.

MATERIALS AND METHODS

Preparation of Cassava and Sweet Potato Roots

About 30 kg each of cassava (cv. Golden Yellow) and sweet potato (cv. BNAS-51) roots were taken from the Philippine Root Crop Research and Training Center experimental plots. They were placed in rattan baskets (40 kg capacity) and stored under laboratory conditions from 0 to 5 days after harvest depending on the treatments. These roots were used in the preparation of the fermentation medium.

Preparation of Rice Starter

The following preparation was made for each fermentation jar. Ninety grams of rice was soaked in a 250 mL Erlenmeyer flask with water for 5 hr. Thereafter, the water was decanted and the flask containing the rice was covered with cotton and sterilized for 15 min at 15 psi. Pure cultures of *Aspergillus oryzae* grown in 10 mL agar slant was inoculated to the soaked rice. The inoculated rice was then incubated for 72 hours at room temperature before using.

Preparation of Yeast Starter

One kg of white potato was peeled, diced and boiled in 2 L water for 30 minutes. The solution was filtered through a linen cloth and the filtrate was collected. Dextrose powder (120 g) was then added to the filtrate and the mixture was heated before distributing 150 mL of the mixture to each 250 mL Erlenmeyer flasks. The flasks were covered with cotton and aluminum foil and sterilized for 15 min at 15 psi. After cooling, pure cultures of *Saccharomyces cerevisiae* grown in 10 mL agar slants were inoculated into the flasks. The starter solution was incubated for 48 hours at room temperature before using.

Alcoholic Fermentation

A mixture of inoculated root crop and water at a ratio of 1:4 was used in all treatments of this experiment. For each experimental sample at indicated periods of processing, about 510 g of roots was used to prepare the fermentation medium. The medium was prepared by grating and cooking the roots for 30 minutes and cooling them. A 90 g prepared rice starter (15% w/w grated roots plus starter) was inoculated to the cooked roots and the mixture was allowed to stand under room conditions for 48 hours. Thereafter, the mash was diluted with 2.4 L water, mixed well, and then transferred to jars of 3.28 liter capacity. The contents of the jars was left undisturbed for another 48 hours for saccharification to occur. Prior to fermentation, the pH of the fermentation liquid was adjusted to 4.5 with 1 N NaOH. A 150-mL prepared yeast starter was then added to the fermentation jar.

Starch and Total Sugar Content Determination

Root samples (100 g each) were collected at indicated periods of sampling, i.e., after grating (initial), saccharification and fermentation. They were oven-dried, ground and analyzed for total sugar and starch contents following the method of Cagampang and Rodriguez (1980).

Alcohol Content Determination

One hundred mL of the fermented liquor was taken from the fermentation jar and distilled. Seventy-five mL of the distillate was mixed with 25 mL distilled water in a 100 mL measuring cylinder. An alcohol hydrometer was placed in the cylinder to measure the percent alcohol on volume/total volume (v/tv) basis. This was done daily for 5 days. The values obtained were used to calculate the alcohol yield per hectare using the following formula:

$$\text{Alcohol yield per hectare (L/ha)} = \frac{\text{Expected alcohol yield (L)}}{\text{Amount of roots used (kg)}} \times \text{Crop yield (kg/ha)}$$

where:

Expected alcohol yield (L) = Wine yield (L) x Percent alcohol in wine, optimum value (in decimal)

Experimental Design

The 6 x 6 factorial experiment was laid out using the completely randomized design (CRD) with three replications. Delay in processing (0, 1, 2, 3, 4 and 5 days after harvest) and duration of fermentation (0, 24, 48, 72, 96 and 120 hours) were used as variables. The Duncan's Multiple Range Test (DMRT) was used in testing significance among treatment means.

RESULTS AND DISCUSSION

Starch and Total Sugar Contents

Late processing significantly affected the starch and total sugar contents of cassava and sweet potato roots at different times of sampling. Regardless of time of sampling, cassava roots which were processed 5 days after harvest gave significantly higher values in both parameters than the other treatments (Table 1). On the other hand, sweet potato roots whose processing was delayed for 4 days had the highest starch content although not significantly different from that of roots processed immediately after harvest (Table 2). The highest total sugar content was obtained from roots processed one day after harvest. Although the starch and sugar contents of the roots differed significantly with each day of delay in processing, no apparent trend was observed.

In cassava and sweet potato, the starch and total sugar contents of the different treatments were significantly affected by time of sampling (Tables 1 and 2). Generally, the starch content decreased after saccharification and this is believed to manifest a number of processes. Kiribuchi and Kubota (1976 as cited by Kawabata et al., 1984) reported that cooking of sweet potato results in very high conversion of starch into maltose. During cooking, the starch grains absorb water, swell and break, and form a gel. Then α -amylase added in the form of rice starter breaks down the starch into

Table 1. Starch and total sugar contents (dry matter basis) of cassava as affected by delay in processing and time of sampling.[1]

Delay in Processing (days)	Starch (%)				Total Sugar (%)			
	Initial	After Saccharification	After Fermentation	Mean	Initial	After Saccharification	After Fermentation	Mean
0	82.42	65.19	40.81	62.81b	0.96	2.74	2.00	1.90c
1	58.04	42.14	38.16	46.11d	1.50	1.97	1.43	1.63d
2	91.43	43.58	50.35	61.79b	1.41	3.23	2.57	2.40b
3	79.77	54.33	44.52	59.54bc	1.50	2.53	1.54	1.86c
4	62.54	52.74	48.26	54.51c	2.81	3.16	0.95	2.31b
5	85.50	58.57	65.70	69.92a	2.80	4.76	3.07	3.54a
Mean	76.62a	52.76b	47.97c		1.83c	3.06a	1.93b	
C.V. (%)		Starch = 7.24				Sugar = 5.20		

[1]In a column or row, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

Table 2. Starch and total sugar contents (dry matter basis) of sweet potato as affected by delay in processing and time of sampling.[1]

Delay in Processing (days)	Starch (%)				Total Sugar (%)			
	Initial	After Saccharification	After Fermentation	Mean	Initial	After Saccharification	After Fermentation	Mean
0	77.57	50.35	41.99	56.64ab	1.82	3.13	2.83	2.59c
1	62.02	37.69	33.75	44.49e	2.38	4.05	3.40	3.28a
2	77.96	52.69	35.33	55.33b	0.95	2.78	2.17	1.97d
3	69.55	50.34	34.65	51.51d	2.60	3.54	3.13	3.09ab
4	85.15	50.74	38.03	57.97a	2.05	4.70	2.64	3.13ab
5	69.01	58.13	34.43	53.86c	2.53	3.10	2.85	2.83bc
Mean	73.54a	49.99b	36.36c		2.06c	3.55a	2.84b	
C.V. (%)	Starch = 2.15				Sugar = 10.72			

[1]In a column or row, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

fermentable sugars before being utilized by the alcoholic yeast. Further decrease in starch content of the roots when sampled after fermentation suggests further breakdown of starch into sugars even during the fermentation process. During fermentation, the fermentable sugars are converted to alcohol, with the amount converted dependent on the amount of fermentable sugars present in the broth, the activity of the fermenting yeast, pH of fermentation medium, and temperature.

In cassava and sweet potato, the increase in sugar content of the roots was not proportional to the decrease in their starch contents (Tables 1 and 2). This is contrary to the expectation that when carefully harvested and undamaged roots are stored under normal conditions, the sugar content of the roots increases while the starch content decreases due to enzymic conversion of starch to sugars as a reponse to mechanical injury. However, other biological and chemical reactions related to this may occur (Uritani, 1982). When sweet potato is harvested by hand, transported and stored; some roots oftentimes suffer mechanical injury. This crop like other root crops readily respond to mechanical injury and show vigorous biological reactions which finally lead to wound healing. In response to mechanical injury, monosaccharide derivatives such as glucose-6 phosphate seem to be used initially as respiratory substrate followed by sucrose, starch, glucose and fructose (Uritani, 1982). Matsushita and Uritani (1974 as cited by Uritani, 1982) claimed that soon after mechanical injury, sucrose is decomposed to glucose and fructose by increased activity of acid invertase. In addition, starch is decomposed to glucose with a lag period of about 12 hrs and this provides the substrate for wound respiration. However, since not all of the roots suffer mechanical injury and injuries are not of the same degree, it is expected therefore that the starch and total sugar contents will vary from root to root and between each day of delay in processing. This is probably the reason why the starch and sugar contents of sweet potato and cassava roots in this experiment did not show any regular trend.

Alcohol Content

Alcohol production was significantly affected by delayed processing in sweet potato (Table 3) but not in cassava (Table 4). However, significant differences in alcohol contents were observed at different fermentation times in both crops. In sweet potato, the highest alcohol yield (3.72% v/tv) was obtained from roots processed after 2 days of delay and lowest yield (2.44% v/tv) from roots processed immediately after harvest. If the highest yield obtained is expressed in terms of the amount of fermentable sugars present in the broth (1.97%, Table 2), then the remaining starch after saccharification (52.69%) must have been saccharified further during

Table 3. Alcohol content of sweet potato as affected by delay in processing and fermentation time.

Delay in Processing (days)	Alcohol Content (%)[1]						
	Fermentation Time (hrs)						Mean
	0	24	48	72	96	120	
0	1.00	1.33	2.67	2.67	3.67	3.33	2.44d
1	1.17	1.33	2.00	5.33	6.00	5.33	3.53b
2	1.00	1.33	3.67	4.67	6.67	5.00	3.72a
3	1.00	1.67	2.33	4.33	4.67	4.33	3.06c
4	1.17	1.83	3.00	3.67	4.33	4.00	3.00c
5	1.00	1.33	2.17	4.00	3.67	3.00	2.53d
Mean	1.06e	1.47d	2.64c	4.11b	4.84a	4.16b	

C.V. (%) = 19.21

[1]In a column or row, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

Table 4. Alcohol content of cassava as affected by delay in processing and fermentation time.

Delay in Processing (days)	Alcohol Content (%)[1] Fermentation Time (hrs)						Mean
	0	24	48	72	96	120	
0	1.50	1.83	2.67	4.00	3.00	1.83	2.47a
1	1.33	1.67	2.17	3.33	3.33	2.00	2.30a
2	1.83	1.83	3.33	3.33	2.33	2.00	2.44a
3	2.17	2.50	3.00	3.67	2.00	2.00	2.56a
4	2.00	2.33	2.67	3.00	2.00	2.00	2.33a
5	2.33	3.00	3.00	2.33	2.00	2.00	2.44a
Mean	1.86e	2.19cd	2.81d	3.28a	2.44c	1.97de	

C.V. (%) = 17.29

[1] In a column or row, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

fermentation thus increasing alcohol production. There was no clear trend, however, to show that the amount of fermentable sugars present in the broth is directly related to the amount of alcohol produced.

In cassava, the initial alcohol yield from roots which were processed 3, 4 and 5 days after harvest was higher than that of roots processed immediately, and at 1 and 2 days after harvest (Table 4). However, as fermentation progressed up to 72 hours, alcohol production in the latter treatments was considerably higher. This could probably be due to the accelerated activity of the enzyme responsible in fermenting the sugars to alcohol. This effect equalized the initial difference in alcohol yield among treatments such that delayed processing did not significantly affect alcohol production. Moreover, alcohol production at 72-120 hours fermentation was more or less of the same level in all treatments. The results suggest that delaying the processing of cassava roots for alcohol production up to 5 days did not statistically decrease alcohol yield.

The peak of alcohol production was attained at 72 hours for cassava and at 96 hours for sweet potato. After these peak periods, alcohol production steadily declined. This result agrees with the findings of Ueda and Koba (1980) that ethanol production rapidly declined after the fourth day of fermentation. The marked decrease in the fermentation rate could be attributed not only to the inactivation of amylase, death of yeast cells but also to accumulation of substances inhibiting alcoholic fermentation by yeast (Ueda and Koba, 1980). Hongo et al. (1967 as cited by Ueda and Koba, 1980) reported the formation of inhibiting substances such as fusel oil (tryptophol, etc.) during alcoholic fermention. Yamashiro et al. (1967 as cited by Ueda and Koba, 1980) identified lower fatty acids as inhibiting substances in the fermented broth.

Results suggest that fermented broth should be harvested after a definite period in order to obtain significantly high alcohol yield. Prolonged fermentation lowers alcohol yield since alcohol is easily affected by inhibiting substances and is readily converted to structurally related compounds.

Alcohol Yield Per Hectare

The alcohol yield per hectare of cassava and sweet potato was calculated based on the percentage of alcohol produced and the total amount of liquid recovered from the fermented material. Table 5 shows that the alcohol yield of cassava per hectare is not significantly affected by delayed processing. This simply suggests that the alcohol yield from cassava roots does not significantly vary even if the roots processed are fresh or stored up to 5 days under ambient conditions. For sweet potato, roots processed 2 days after harvest gave the highest alcohol yield which was significantly different from the rest of the treatments.

Table 5. Alcohol yield per hectare of cassava and sweet potato as affected by delayed processing.

Delay in Processing (days)	Alcohol Yield Per Hectare (L/ha)[1]	
	Cassava	Sweet Potato
0	2856.43a	3302.20c
1	2399.43a	5292.10ab
2	2385.13a	5929.27a
3	2607.27a	4088.80bc
4	2174.07a	3725.10c
5	2217.40a	3565.87c
C.V. (%)	15.53	17.08

[1]In a column, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

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