

# EFFECT OF PECTINASE, CELLULASE AND NUTRIENT ADDITION ON FERMENTATION OF SWEET POTATO MASHES

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## ABSTRACT

Addition of either cellulase or pectinase mixture to pre-heated sweet potato mashes at the time of yeast inoculation increased ethanol production up to twice that of untreated mashes, possibly by enhancing the post-heating activity of the native amylase and/or by lowering the viscosity of the mash, thus allowing better mixing and providing more chances for amylase attack on starch granules. With enzyme addition, heating mashes beyond 60°C did not increase ethanol yield. However, ethanol production was higher if enzymes were not added. The use of a common yeast nutrient supplement, corn steep, was detrimental to fermentation.

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**KEY WORDS:** Sweet potato [*Ipomoea batatas* (L.) Lam] genotypes. Fermentation. Prefermentation heating. Pectinase. Cellulase. Corn steep. Ethanol yield.

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## INTRODUCTION

Because of its calorie yield, sweet potato [*Ipomoea batatas* (L.) La] appears to have considerable potential as an industrial raw material for fuel ethanol production (Azhar, 1981) especially in tropical regions (Bouwkamp, 1984). Recently, more attention has been given to sweet potato fermentation methods

(Azhar and Hamdy, 1981; Chua et al., 1984; Matsuoka et al., 1982; Sreekantiah and Rao, 1980; Ueda and Koba, 1980) which include studies on the use of non-traditional enzymes and mash preparations as aids in fermentation. For instance, native sweet potato beta-amylase which is active at high temperatures (60-70°C) may be used to partially saccharify sweet potato slurry

(Hoover and Harmon, 1967; McArdle and Bouwkamp, 1986).

Prefermentation treatments for traditional raw materials usually include heating of the mash to 100°C for starch hydration and gelatinization. Prefermentation heating of sweet potato mashes containing native beta-amylases to relatively lower final temperatures (60-70°C) may be advantageous to small-scale producers because less total energy is required and waste heat from distillation and cooling of spent mash can be recovered. Experiments were conducted to investigate the feasibility of the use of low temperature, enzymes and nutrient in the production of ethanol from sweet potato.

## MATERIALS AND METHODS

Plants of two sweet potato genotypes, 'Rojo Blanco' and 'MD 715' (a University of Maryland breeding line), were grown in replicated plots at the Salisbury, Maryland vegetable research facility. These lines were chosen because of their high yield and high root dry matter. Roots were harvested after 120 to 130 days and were stored at 13°C until use.

The enzyme preparations "C-1" cellulase and "Super-Pec" pectinase were obtained from the International Enzyme Company, Inc., Chester, New Hampshire, U.S.A. Yeast inoculum (15% w.w.) was prepared from compressed yeast (*Saccharomyces cerevisiae*) cake and distilled water (dw).

The nutrient material was corn steep (or corn steep water) furnished by Cargill, Inc. Corn steep is the water drained from corn soaked for softening prior to starch separation in wet milling. It is often concentrated to approximately 50% solids and used as a nutrient source in large-scale fermentations (Johnson and Peterson, 1974). The material used in this experiment had solids concentration of 47.5% and nitrogen concentration (dwb) of 5.83% (determined by semi-micro Kjeldahl method).

### *Mash Preparation, Enzyme and Nutrient Treatment, and Fermentation*

*Experiment 1.* Mashes of 'Rojo Blanco' were made by diluting roots ground through a 0.76 cm screen with an equal weight of distilled water and simultaneously adjusting the pH to 5.0 with HCl. Three mash replicates were heated to each of the following temperatures: 30, 40, 50, 60, 70 and 80°C. This initial increase in temperature was accomplished as quickly as possible at a constant rate (10°C/minute). From each mash, 1.5 kg portions were decanted into 2-liter Erlenmeyer flasks and cooled to 25°C in a running water bath as soon as the desired temperatures were reached. One flask in each temperature treatment served as control and another was inoculated with 5 g pectinase and 5 g cellulase in 10 ml dw. All flasks received 5 ml yeast inoculum. The flasks were fitted

with airlocks and were left to ferment at 30°C for 4 days.

*Experiment 2.* Mashers of 'Rojo Blanco' and 'MD 715' were prepared as before except that the temperatures of all mashers were raised to 80°C initially. Flasks were inoculated with one of the following solutions: 1) 10 ml dw, 2) 5 g pectinase in 10 ml dw, 3) 5 g cellulase in 10 ml dw, and 4) 5 g pectinase + 5 g cellulase in 10 ml dw. The mashers in all flasks were inoculated with yeast and fermented as in experiment 1.

*Experiment 3.* Mashers of 'Rojo Blanco' were initially prepared as in experiment 2. Twelve flasks were inoculated with 5 ml yeast inoculum, and 5 g pectinase + 5 g cellulase in 10 ml dw. One third of the flasks received corn steep at 5% of their weight, one third at 2.5%, and one third at 0%. The flasks were fitted with airlocks and were left to ferment as before.

*Ethanol Determination.* Ethanol was determined using the method of Davis and Chace (1969) except that an amount of saturated  $(\text{NH}_4)_2\text{SO}_4$  equal to that of the mash extract was added to the test flasks. Loss of dry matter was also used as an indirect measurement of ethanol formation.

*Alcohol Insoluble Solids Determination.* To determine alcohol insoluble solids (AIS), a 25 g mash sample was subjected to boiling in 80% (v/v) ethanol, allowed to settle for 12 hours, filtered through Whatman #1 filter paper, and

weighed after drying in a 75°C oven for 12 hours.

*Fermentation Rate.* In flasks for experiment 3, fermentation rate was determined at 10, 25 and 33 hrs after inoculation. This was measured as the rate of gas formation (bubbles exiting the flasks) per minute.

## RESULTS AND DISCUSSION

### *Effect of Prefermentative Heat Treatments on Enzyme Activity*

Regression analysis of experiment 1 data revealed that enzyme treatment increased mash ethanol concentration by 100% (averaging 5.4% ethanol in treated flasks compared to 2.7% ethanol in untreated flasks). In addition, enzyme effects might have been influenced by prefermentation heat treatment. The regression equations calculated for the enzyme and the non-enzyme treatments (Fig. 1) were quadratic and linear, respectively. It appears that without the addition of cellulase and pectinase to fermenting mashers, ethanol concentration increased linearly with respect to prefermentation temperature. When the enzymes were added, heating beyond 60°C did not increase ethanol production.

### *Effects of Enzyme Treatments on Ethanol Production of Mashers of Two Sweet Potato Cultivars*

As in the first experiment, addition of prepared commercial enzymes to sweet potato mashers at

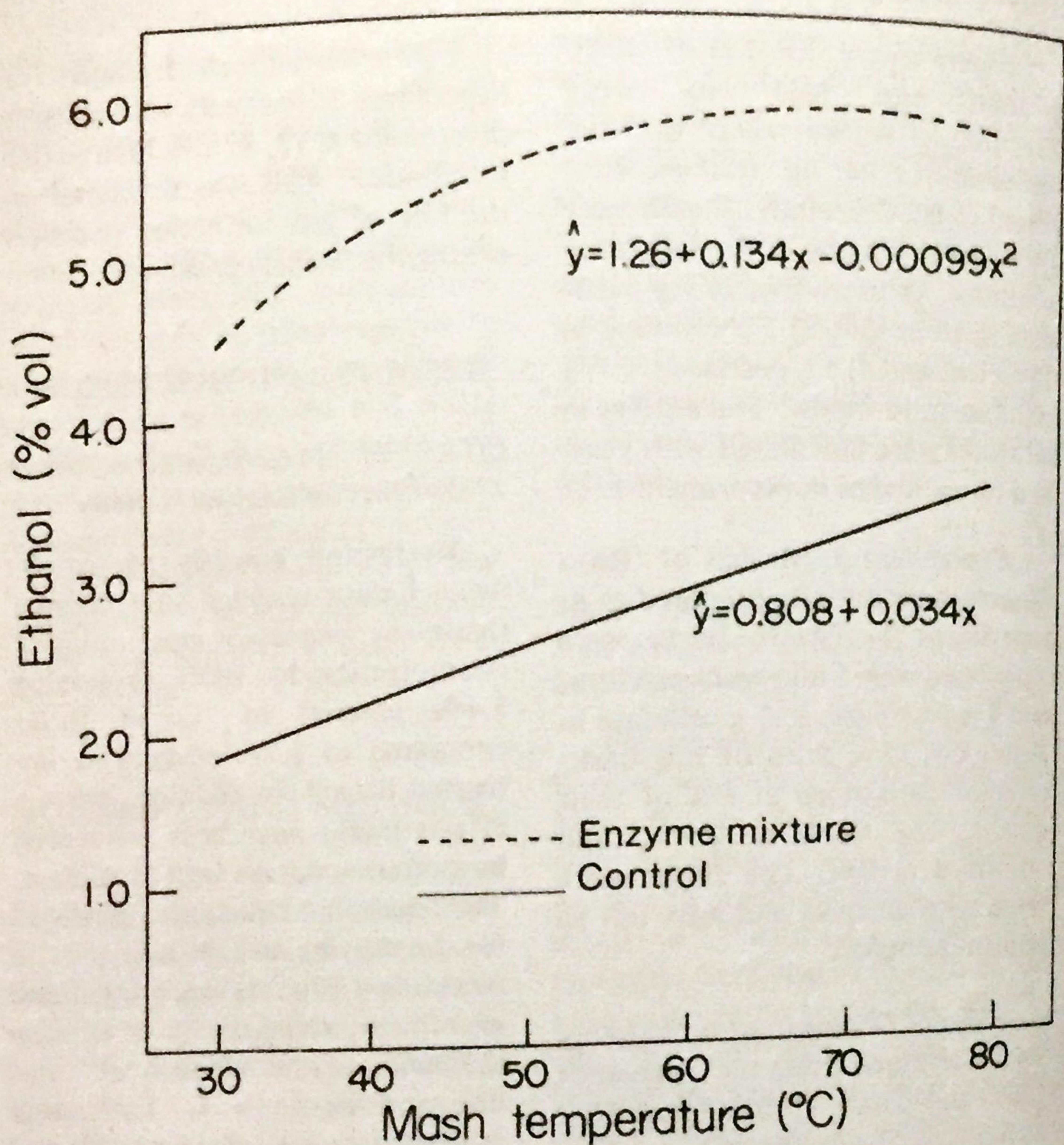


Figure 1. Effect of enzyme addition to sweet potato mashes with increasing preheat temperatures.

the time of yeast inoculation generally increased the production of ethanol.

The addition of both enzymes to fermenting mashes of either 'Rojo Blanco' or 'MD 715' significantly increased ethanol production, measured directly or by loss of mash

dry matter, over that in untreated controls (Table 1). Change in mash dry matter of both genotypes, suggests that pectinase addition was more efficacious than cellulase addition, but it had statistically similar effect as the addition of both enzymes in promoting ethanol produc-

**Table 1.** Effects of enzyme treatments on ethanol yield, dry matter, and alcohol insoluble solids of mashes of two sweet potato genotypes. <sup>1</sup>

Treatment	'Rojo Blanco'			'MD 715'		
	EtOH <sup>2</sup>	DM <sup>3</sup>	AIS <sup>4</sup>	EtOH	DM	AIS
Initial (unheated)	0	100.0	67.3	0	100.0	59.8
Heated						
No enzyme	66.7b	55.5c	29.1c	51.0c	41.7c	25.6c
Cellulase	77.2ab	33.7b	15.6b	47.8c	31.0b	15.3b
Pectinase	71.4ab	30.3a	17.3b	62.7b	28.0a	14.0ab
Cellulase + Pectinase	81.3a	27.7a	13.6a	69.2a	28.6a	13.2a

<sup>1</sup>Means within a column followed by a common letter are not significantly different at 5% level, DMRT.

<sup>2</sup>EtOH = ethanol yield as percentage of the theoretical maximum that could be obtained from the mash dry matter (based on 100% starch content).

<sup>3</sup>DM = mash dry matter expressed as percentage of the original mash dry matter.

<sup>4</sup>AIS = mash alcohol insoluble solids expressed as percentage of the original mash dry matter.

tion. This relationship was not as clearly shown by the direct ethanol measurements. Based on both measurements, 'Rojo Blanco' mashes fermented to a greater extent than 'MD 715' mashes with or without enzyme treatment ( $P < 0.05$  for main effect of genotype).

The relatively large increases in fermentative activity in these mashes following cellulase and pectinase addition is unlikely to be due solely to the breakdown of their respective substrates, cellulose and pectic material. The pectic and cellulosic fractions of sweet potato amount to 7-8% of dry weight according to Sistrunk (1977). Assuming complete

yeast utilization, complete hydrolysis of these fractions and ethanolic fermentation of the hydrolytic products would perhaps account for only 15% increase in ethanol (by volume).

The enzyme treatment possibly increased the efficacy of residual native amylase activity during fermentation. This is supported by the close correspondence of AIS and dry matter (DM) figures in both mash types (Table 1). Inasmuch as the major portion (61-72%) of sweet potato AIS is starch (Scott and Matthews, 1957), constancy of the AIS/DM ratio suggests that the starch fraction was responsible for

differences in ethanol production by the various treatments.

Svendsby et al. (1981) showed that combination of glucoamylase and pectin depolymerase (polygalacturonase) treatments on minced sweet potato roots increased the rate of production and final concentration of reducing sugars by more than 40% over those without pectinase. Pectinase alone accounted for a very small increase in reducing sugars. The authors did not attempt to explain the increase, but noted that viscosity of the mash decreased with pectinase addition. This lowering of viscosity was also noted in the enzyme-treated flasks in experiments 1 and 2. A reduction in viscosity may permit better mixing of the mash, providing more chances for amylase attack on starch granules. As for the former explanation, this would undoubtedly create an increased enzyme effect at lower temperatures, where less amylase denaturation would occur. This interaction was apparent in experiment 1 (Fig. 1).

#### *Effects of Nutrient Addition on Fermentation.*

Experiment 3 which was designed to show the effects of added yeast nutrients to sweet potato mash, provided unexpected results. The addition of 2.5% and 5% corn steep

to 'Rojo Blanco' sweet potato mashes resulted in lowered fermentation rate and final production of ethanol whether measured directly or using change in dry matter.

Ethanol concentration and change in dry matter decreased linearly with increasing concentration of nutrient material (Fig. 2). These decreases could be described by significant linear regression equations. Rate of fermentation, as measured by bubble counts of the fermenting flasks, was also similarly depressed by nutrient addition (Table 2).

The lowering of ethanol production and production rate with the addition of a potential yeast nutrient solution is perplexing. It seems unlikely that a simple nutritional toxicity could be at fault. A 5% corn steep addition would increase the nitrogen level of a 50% aqueous sweet potato slurry from roughly 0.6% to 0.9% but this seemed an insignificant amount. There might be vitamins or other compounds which were ordinarily beneficial to yeast growth but were present in toxic amounts; and this requires further study. It appears, for yet unknown reasons, that this type of nutrient material should not be added in sweet potato fermentations unless evidence for its benefit is found.

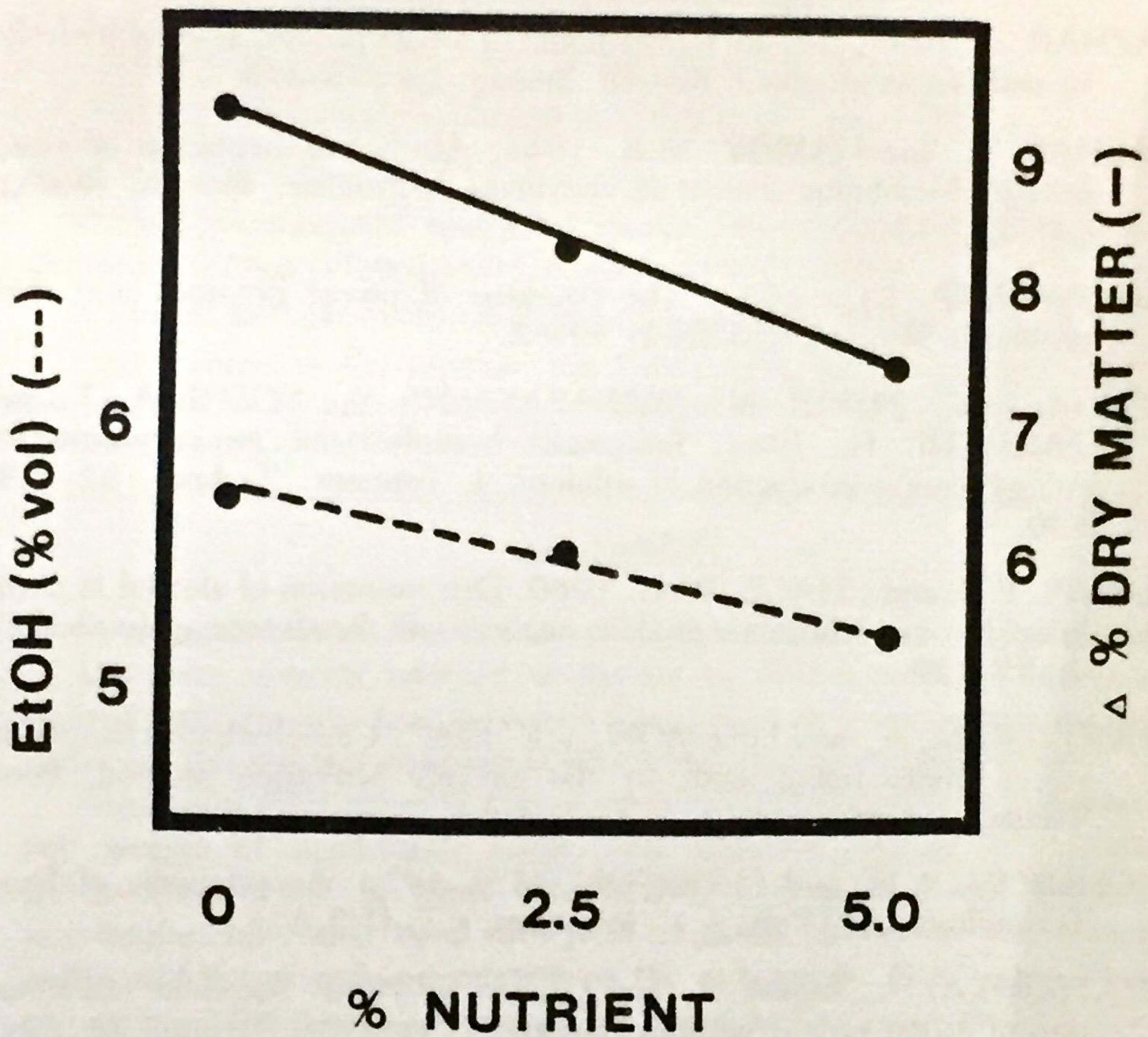


Figure 2. Influence of nutrient addition on ethanol production in 'Rojo Blanco' mashes.

Table 2. Fermentation rate of sweet potato mashes with three levels of nutrient addition at different times of sampling.<sup>1</sup>

Level of Nutrient Addition	Fermentation Rate (gas bubbles/min)		
	10 hours	25 hours	33 hours
0	49.3a	30.0a	11.8a
2.5%	30.3ab	16.0a	7.3a
5.0%	11.5b	7.0b	6.5a

<sup>1</sup>Means within a column followed by a common letter are not significantly different at 5% level, DMRT.

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