A METHOD FOR RAPID EXTRACTION OF SUGAR FROM CORN STALK TISSUE

H. O. BETTERTON AND A. J. PAPPELIS
University of Maryland and Southern Illinois University

ABSTRACT. — A three minute homogenization of corn pith tissue killed in 80 per cent ethanol and stored for twenty four hours, extracted about 99 per cent of the total sugars. This high level of extraction was consistent in five sampling dates beginning before tassle elongation and ending two months after silking. Centrifugation of the lead precipitate obtained during the clearing stage of sugar analysis decreased the time required per sample by elimination of filtration and washing of the precipitate.

Traditional methods for extracting sugar from plant tissue involve laborious procedures using either a Soxhlet extractor or repeated extraction of the tissue with 80% ethanol, decantation of supernatants, and combination of resulting fractions (Association of Official Agricultural Chemists 1945, 1960; Loomis, 1926, 1935; Paech and Tracey, 1955). Thomas, et al (1949) have reported that sugar can be extracted from a wide variety of plant tissues by grinding them in a Waring Blendor with 200 ml of 70% ethanol for seven minutes. This procedure was reported to be as good as the A.O.A.C. method. However, Thomas, et al sampled young succulent plants and no evidence was presented that sugar could be extracted as easily at other stages in the life cycle. Furthermore, they did not work with corn stalk tissue. Pappelis (1957) found that more than 97% of the reducing sugars (measured as glucose) and nearly 100% of the sucrose (sucrase hydrolyzable fraction) could be extracted from entire internode tissue of mature corn killed in 80% ethanol. stored for four to six months and homogenized for four minutes in fresh, hot ethanol using an Omnimixer. Chemical analysis of aliquots from combined filtrates and original killing fluid and from the extracts of the residues (collected after 36 hours of Soxhlet extraction) were used to determine the per cent extracted by the Omnimixer. The length of time required after killing to obtain this level of extraction was not ascertained. This paper presents data to: determine the time required after killing for a high level of extraction using a three minute homogenization period; eliminate the filtration step in clearing; and, determine the reliability of this method for extraction of sugars from stalk pith tissue at various stages of maturity.

MATERIALS AND METHODS

Seeds of corn (*Zea mays* L.) var. Wf9x38-11 were planted on the Southern Illinois University Agronomy Farm May 21, 1962. Samples were taken at five times during the growing season and represented various stages of growth. Approximately 50% of the plants had one or more inches of silks exposed on July 30. Stalk samples, always collected at noon, consisted of the first through the sixth fully elongated internodes above the uppermost brace roots. The six internodes were removed to exclude all nodal tissue and a longitudinal cylinder of pith tissue was

TABLE 1.- Per cent of sugar extracted by use of the Waring Blendor.

Date of sample collection	Hours after killing	Per cent recovery		
		Reducing sugar	Sucrose	Total sugar
July 10[1]	0	99.0	100.0	99.4
	24	100.0	96.5	99.6
	48[3]	100.0	98.2	99.6
July 22[1]	0	98.0	98.5	98.1
	24	99.2	100.0	99.3
August 7[2]	0	98.4	99.8	99.5
	24	98.0	100.0	99.6
Sept. 6[2]	0	95.6	99.8	99.0
	24	98.0	99.6	99.4
Oct. 7[2]	0	96.0	99.6	99.4
	24	97.0	99.6	99.2

[1] Average of 6 Replicates.
[2] Average of 5 Replicates.
[3] On subsequent sampling dates, 48 and 72 hour extractions were not significantly greater than the 24 hour extractions.

removed along the central axis using a cork borer 1 cm in diameter. Each cylinder was cut to 6 cm lengths, and then into six pieces, each 1 cm in length. Each of these six pieces were placed into separate storage jars together with 50 ml of boiling 80% ethanol. This procedure provided for samples of tissue from the upper to the lower internodes in each of the six jars. It was repeated with tissue from additional plants until each of the six jars contained from 10 to 15 segments. Twenty-four such jars were prepared on each date. Six of the jars were selected randomly for each treatment in which the tissue was homogenized immediately, 24, 48, or 72 hours after killing. In each, a quantitative transfer was made into a Waring Blendor with a final volume of about 150 ml of 80% ethanol (room temperature). The tissue was homogenized at high speed for three minutes with a stop after one or two minutes to allow the sides of the blendor jar to be washed down with more alcohol. The slurry was filtered through Whatman No. 1 filter paper on a Buchner funnel. The blendor jar and residue were rinsed five times with 80% ethanol, and the filtrate so obtained was returned to the original sample jar after dilution to a standard volume. The tissue residues and filter paper were placed in a Soxhlet extractor with 80% ethanol and extraction allowed to proceed for 36 hours. Soxhlet extracts were collected and diluted to volume.

Suitable aliquots of ethanol from the homogenized sample or Soxhlet extract were evaporated almost to dryness, diluted to 50 ml with distilled water, and cleared according to the method described by Loomis (1926). After 10 minutes, 10 ml of dibasic potassium phosphate (125 g per liter) were added to precipitate the excess lead and the solution was brought to a volume of 100 ml. The precipitate was removed from suspension by centrifugation at 3000 rpm for four minutes.

Reducing sugars were analyzed ac-

cording to the method described by Hassid (1937), and are reported as glucose. Sucrase was prepared according to the method described by Loomis and Schull (1937) and, following hydrolysis, added reducing sugar was determined and calculated as sucrose.

Experimental Results and Discussion

The data for sugar extraction are presented in Table 1. The grinding procedure extracted over 99% of the total sugar after 24 hours in the alcohol. The extraction never exceeded 99.6% for total sugar, even at 72 hours after killing. Evidently, there was a small amount of sugar which could not be extracted by grinding and rinsing of the residues on the funnel that could be removed from the residues by 36 hour Soxhlet extraction. This high degree of extraction was consistent throughout the study which included young succulent tissue of the stalk prior to tassle formation to dry stalk samples.

The advantage of this system over others lies in the reduced amount of extraction and handling time required per sample. In addition, collection and killing of tissue in routine studies with many samples can be followed in 24 hours by the extraction step.

Although the evaporation of alcohol from sample aliquots was completed as described, another time-saving step was incorporated in the clearing procedure. Filtration and washing of the lead phosphate precipitate obtained from deleading, which required much bench space and time, was eliminated by centrifugation of the precipitate. The pellet of the precipitate did not include any Soxhlet extractable sugar and occupied less than 1 ml of volume. The clear supernatent was decanted and aliquots for reducing sugar and sucrase hydrolyzable sugar were processed according to the normal procedure.

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